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PROCEEDINGS *of the* TENTH RESEARCH CONFERENCE

Sponsored by

THE RESEARCH ADVISORY COUNCIL

of the

AMERICAN MEAT INSTITUTE FOUNDATION

at

THE UNIVERSITY OF CHICAGO

MARCH 27 AND 28, 1958

CIRCULAR No. 45 • JULY, 1958

Issued by

AMERICAN MEAT INSTITUTE
FOUNDATION

939 EAST FIFTY-SEVENTH STREET
CHICAGO 37, ILLINOIS

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FOREWORD

Research conferences for scientific and technical personnel in the meat packing and allied industries and for experiment-station, university, and government scientists interested in scientific aspects of the production of livestock and the processing and utilization of products derived from livestock have been held annually since 1949 on the University of Chicago campus.

The first nine conferences were sponsored by the Council on Research of the American Meat Institute. The Tenth Research Conference, for the benefit of the same people and with the same objectives as previous conferences, was sponsored by the Research Advisory Council of the American Meat Institute Foundation.

To avoid confusion, this Tenth Conference and all future ones should be considered the continuation of this series of meetings, even though the sponsoring organization has changed.

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TABLE OF CONTENTS

		Page
I.	Hot-Melt Meat Coatings—W. M. Westveer, The Dow Chemical Company, Midland, Michigan	1
II.	Irradiation for the Meat Industry—W. M. Urbain, Research Laboratories, Swift & Company, Chicago, Illinois	7
III.	Fresh-Meat Characteristics Which Influence Packaging Requirements—M. C. Urbin and G. D. Wilson, American Meat Institute Foundation, The University of Chicago, Chicago, Illinois	13
IV.	Packaging to Meet Consumer Demands—Glen R. Curtis, Independent Grocers Alliance, Chicago, Illinois	21
V.	Dietary Fats, Blood Fats, and Atherosclerosis—Laurance W. Kinsell, M.D., Institute for Metabolic Research, Highland-Alameda County Hospital, Oakland, California	25
VI.	Fat Metabolism and Energetics—E. P. Kennedy and W. Wallace Cleland, University of Chicago, Chicago, Illinois	29
VII.	Growth-promoting Properties of Meat Meal—C. J. Ackerman, Virginia Polytechnic Institute, Blacksburg, Virginia	35
VIII.	Influence of Preslaughter Feeding and Holding on Meat Quality—Marshall C. Heck, University of Arkansas, Fayetteville, Arkansas	41
IX.	Tranquilizers in Animal Feeds—H. G. Luther, Chas. Pfizer & Co., Inc., Agricultural Research & Development Dept., Terre Haute, Indiana	47
X.	Analytical Variations in Sausage Composition—H. P. Furgal, Armour and Company; R. H. Harper, Swift & Company; C. D. Lacey, Wilson & Co., Inc.; F. C. Olson, Oscar Mayer & Co., Chicago, Illinois	59
XI.	Meat Tenderizing by Enzymes—Vincent S. Bavisotto, Paul-Lewis Laboratories, Inc., Milwaukee, Wisconsin	67
XII.	Influence of Sires on Tenderness of Beef—T. C. Cartwright, O. D. Butler, and Sylvia Cover, Texas Agricultural Experiment Station, College Station, Texas	75
XIII.	Pigment-Antioxidant Relationships to Meat-Color Stability—W. Duane Brown and A. L. Tappel, University of California, Davis, California	81
XIV.	Some Factors Influencing Thermal Resistance of Bacterial Spores—Z. John Ordal and Richard V. Lechowich, University of Illinois, Urbana, Illinois	91

I

HOT-MELT MEAT COATINGS

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The packaging industry has grown from a relatively minor position to one of the largest industries in our country today. Included in this broad development has been the packaging of meat and meat food products.

The packaging of frozen meats is a relatively new field and many different types of wrap and package are in use. Among these may be included cellophane, Cryovac, Pliofilm, Saran, Mylar, polyethylene, boxboard, coated paper, foil, and others. All these materials have one shortcoming in common: they fail to give skintight contact. Ice crystals develop in the air pockets between the wrap and the meat; these crystals give evidence of dehydration and also cause a loss in the transparency of clear films. A suggested remedy for this problem is a properly formulated hot-melt coating which will give skintight contact and retain full transparency.

In the early development work with hot melts, wax coatings were formulated. They are still being used to some extent today. Frozen meat is dipped in the wax, and skintight, good coverage usually results. However, wax coatings tend to be brittle at low storage temperatures, and breakage of the coating permits dehydration of the meat. Another disadvantage in the use of wax is its opacity. These disadvantages of wax coatings have encouraged further development work with other types of hot melts.

Figure 1

One of the new hot-melt coatings being developed by The Dow Chemical Company has many advantages when compared to present packages used for the protection of frozen meat. Before we get into the properties of this particular coating, let us first briefly discuss the coating itself, how it may be applied, and how it protects the frozen meat.

Table 1 summarizes some of the advantages of this experimental meat coating. As can be seen, the coating is clear, transparent, tough, and easily removed by peeling. Also the quality of the meat at the time of packaging is adequately maintained by this coating if the sample is properly stored (i.e., 0^{o}F. or below). This will be discussed in more detail later. The coating is skintight, impermeable to moisture (thereby preventing dehydration), protects the meat from shopper contamination, is flexible at freezer temperatures, and is readily adaptable to a completely automatic packaging process because of short setup time.

TABLE 1

Advantages of a Hot-Melt Coating

1. Clear, transparent, and tough
2. Easily removed by peeling
3. Maintains original quality of meat
4. Skintight
5. Impermeable to moisture
6. Protects meat from shopper contamination
7. Flexible at freezer temperature
8. Adaptable to automatic process

There are two methods by which this material can be applied to meat: one is by a hand-dip method, the other by machine application. We will discuss the hand-dip method first.

Figure 2

The hand-dip method has been used by us to determine whether or not a given formulation has promise for future use. Because of the simplicity of this method, it may have commercial utility. The meat to be coated is threaded with string for convenience in handling and is then prefrozen by any of the conventional freezing methods. The frozen meat is dipped into a hot melt of the formulation maintained at 325°F. The sample is immediately withdrawn from the hot melt and allowed to drain for a few seconds. After 15-30 seconds, the coated sample can be handled and placed back in the freezer. The thickness resulting from this type of operation, when a hot melt with a Brookfield viscosity of 185 cps at 325°F. is used, is approximately 50 mil.

The machine method of application is still in the development stage. Early trials have shown that this method applies a thinner coating, resulting in greater economy and a faster operation.

A little more than a year ago a co-operative study was undertaken with Michigan State University to determine the effectiveness of the hot-melt coatings in preserving the quality of meat stored for a year at both 0°F. and -20°F. Samples of hand-dip-coated meat were stored directly in front of a blower in a blast freezer.

As can be seen in Table 2a, there was no weight loss on the samples of meat stored in this manner. These tests reveal the ability of this coating to prevent dehydration of frozen meat. Other tests run in our own laboratory show that a 20-mil film can give very similar results. The samples of meat stored for a year at Michigan State University still had satisfactory color. A bacteriological study at M.S.U. showed a significant reduction in bacterial count when inoculated, coated steaks were compared to control steaks. However, bacterial counts on the coated meat obtained at a later date showed an increase in population, indicating that the initial reduction was only a pasteurizing effect.

TABLE 2a

Results of Tests at M.S.U.

1. No weight loss in 12 months (+0.23%)
2. Munsell color notations showed little change on stored samples
3. Bacterial counts

	Inoc.	Control
0 months	72,000	135,000
6 months	350,000	670,000

Taste tests conducted according to a procedure developed by the Quartermaster Corps were conducted on coated beef steaks and pork chops stored for 1 year and on coated pork sausage stored for 11 months. All pieces of meat proved to be acceptable. TBA tests for rancidity[1] were made with both pork chops and pork sausage. The results of these tests verified the findings of the taste panel.

Oxygen transmission rates were conducted by ASTM method D1434-56T. The results are given in cubic centimeters per hundred square inches per 24 hours per atmosphere. At −20°F., with a 50-mil film, the transmission rate

1. TBA determination for rancidity in pork (Food Tech., 8:326, 1954).

TABLE 2b

Results of Tests at M.S.U.

4. Taste Tests:

	Pork		Beef		Sausage
	Lean	Fat	Lean	Fat	
0 months	6.5	5.6	6.6	5.3	6.5
12 months	6.9	5.2	7.0	4.9	7.2

5. TBA Values:

	Pork	Sausage
0 months	0.272	0.154
12 months	0.491 (fat only)	0.358 (11 months)

was 7 cc., while the rate with a 20-mil film was about 26 cc. Similar tests run at room temperature (73°F.) indicate a transmission rate of 83 cc. for a 50-mil film. At room temperature these results compare with .56 cc. for 1-mil Saran and 920 cc. for a 1-mil polyethylene film.

In the formulation of these hot melts there is a definite relationship between melt viscosity and temperature. Brookfield viscosities were run at 30 rpm with a No. 2 spindle at temperatures from 300° to 340°F. Graph I shows a reduction in viscosity as the temperature increases, and this seems to be pretty much of a straight-line function.

The relationship between temperature and the grams of coating picked up on a dipping operation is shown in Graph II. As can be seen, the amount of coating picked up lessened as the temperature increased. However, this function seemed to begin leveling off at about 330°F. As a result, we maintain the melt at 325°F. during a dip coating.

Most of our work to date has been hand-dip coating of frozen beef, pork, lamb, and veal. Preliminary hand-dip coating of fresh, unfrozen meat is being investigated for commercial use. We intend also to investigate the machine coasting of fresh, unfrozen meat.

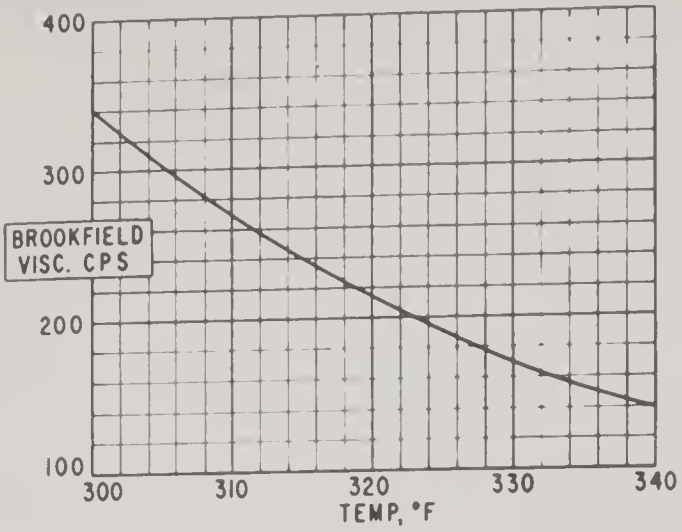
A very limited amount of work with smoked meats shows promise for the hot-melt coating. However, because of the special requirements of this type of meat, further formulating work may be necessary. Future work will involve both hand-dip and machine coating of smoked meats, poultry, and fresh, unfrozen meat.

TABLE 3

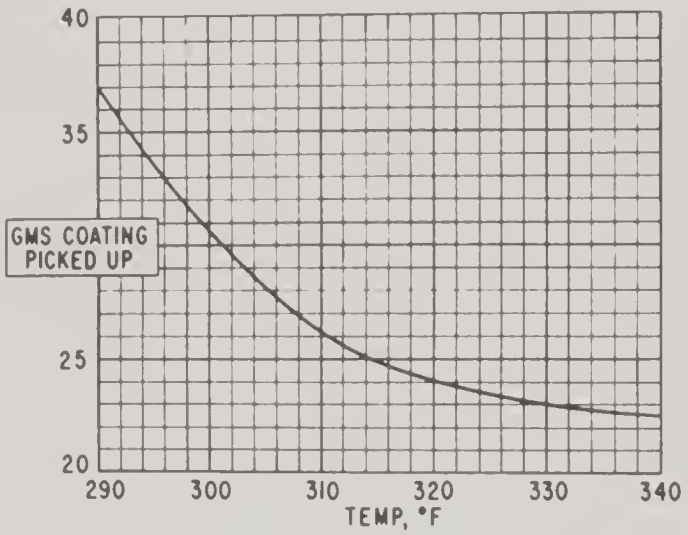
Oxygen Transmission Rates*

Temp.	Hot Melt		Saran	PE
	50 mil	20 mil	1 mil	1 mil
-20°F.	7 cc.	26 cc.	lt 0.01	-
+73°F.	83 cc.	-	0.56 cc.	920 cc.

*cc./100 $in.^2$/24 hrs./atm.



Graph I. Viscosity versus temperature.



Graph II. Temperature of melt versus coating pick-up.

ACKNOWLEDGMENT

We wish at this time to acknowledge the work done by Professor Lyman Bratzler and Dr. Al Pearson of Michigan State University in the co-operative study of this meat-coating project.

II

IRRADIATION FOR THE MEAT INDUSTRY[1]

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As an introduction it can be noted that early work on radiation preservation was the effort of some laboratories both in and outside the meat packing industry. While such activity continues today, the major effort in this country is now under the sponsorship of the U.S. Army Quartermaster. Hence the major work is being carried out with military objectives, which may not always coincide with civilian purposes. The viewpoint of this paper is entirely that of the packer toward the civilian market. Where information is used that has been developed as a result of Quartermaster-sponsored research, it is interpreted in terms of civilian rather than military usage. Obviously, such interpretation as is presented is chargeable solely to the author and should be so regarded.

In view of the large amount of attention that has been given to the subject in recent years, it is not very likely that there is anyone who has not heard of the proposed use of irradiation for food preservation. In view of this, a description of the process in detail does not seem necessary. It is my plan, therefore, rather to provide an appraisal of the current state of our knowledge and to draw some conclusions regarding the future.

Irradiation utilizes ionizing radiation to destroy food-spoilage micro-organisms and thereby to preserve the food. While the physicist classifies a number of radiations as ionizing, only two appear to be of practical significance in the treatment of foods. One is the corpuscular radiation made up of electrons and called, variously, "electron beams," "cathode rays," or "beta rays"—the precise terminology usually indicating the source of the radiation. However, for food treatment, the principal source of this kind of ionizing radiation is machines, and perhaps the term "electron beams" is most suitable. The other type of ionizing radiation for treatment of foods is commonly referred to as "gamma radiation." This, it will be recalled, is an electromagnetic radiation of the same character as X-rays.

The two types of ionizing radiation—electron beams and gamma rays—appear to be essentially the same in their bactericidal action. Based on evidence available today, one would conclude that any difference in action which may exist is small. There is, of course, a fundamental difference in the penetrability of these two radiations. Gamma rays, in general, are very penetrating and because of this characteristic are particularly useful in treating thick

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1. This paper reports research undertaken in co-operation with the Quartermaster Food and Container Institute for the Armed Forces, Quartermaster Research and Engineering Command, U.S. Army, and has been assigned number 850 in the series of papers approved for publication. The views or conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

objects. This is especially true when it is desired that every volume element of the food receive treatment. The penetration of electron beams, on the other hand, is dependent upon the energy of the electrons used. The faster the electron is made to move, the higher the energy it carries and the greater the depth of penetration. Hence an energy suited to the purpose in mind can be selected. If, for example, surface treatment is desired, a relatively low energy can be used and the electrons absorbed essentially on the surface.

Various sources of ionizing radiation are available today. As stated previously, machines are the usual sources contemplated for generation of electron beams for treating foods. Each of the various types of machines depends upon the fact that the electron carries a negative electric charge and makes use of the acceleration imparted to the electron by an electrostatic field. There are, in the main, three basic types of electron-beam generators which can be considered: (1) the resonant transformer; (2) the Van de Graaff generator; and (3) the linear accelerator. The first two units, while very efficient and capable of producing large numbers of electrons, have definite limitations as to the energy which they can impart. One would judge that a practical limit of approximately 5 million volts exists for these machines. This limit, translated into thickness capable of being treated, amounts to about 1 inch, with the radiation being applied from two sides. The linear accelerator, on the other hand, can produce quite higher voltages. For example, the linear accelerator which is being built for the Army Ionizing Radiation Center at Lathrop, California, is expected to provide 24-million-volt electrons. The linear accelerator designed for food irradiation is the newest of the three electron generators to become available. One can conclude, therefore, that there are electron-beam generators available today which can be built or selected for the type of radiation process contemplated in any particular instance.

For gamma-ray sources we must look to radioactive materials which result from the operation of an atomic reactor. The gamma rays originate from the radioactive disintegration of a suitable element which has become radioactive as a result of the action of the reactor. For experimental purposes two kinds of radioactive isotopes have been utilized. The first was cobalt-60, which produces a strong gamma-ray emission. Later, the fuel elements from atomic reactors became available. These contain radioactive fission products from uranium, and, while they are a mixture, the fuel element in toto is an intense source of gamma radiation. It is because of this high intensity that much of the experimental work has been done with fuel elements. At the present time there is no commercial installation using gamma radiation for production purposes.

A consideration of how ionizing radiation can be utilized in treating foods quickly suggests two principal methods: (1) the destruction of all food-spoilage micro-organisms present, leading to a sterile product, and (2) the destruction of a sufficient number of food-spoilage micro-organisms to produce an extension of product life under some chosen conditions. These two methods, which, for want of better terms, have been called "sterilization" and "pasteurization," respectively, have separate utility and separate problems.

Let us turn our attention first to sterilization by radiation and let us confine our attention to meat. Among the first important information needed in connection with this process is a knowledge of the amount of radiation required to produce sterility. Strangely, this is not known with a high degree of precision at this time. It is known that the radiation sensitivity of various bacteria is different. In general, spores are much more radiation resistant than are vegetative forms. Apparently, the most radiation-resistant micro-organism commonly occurring in meat is Clostridium botulinum. The best estimate at the present for the sterilization requirement for this micro-organism using radiation alone in a non-acid, low-salt medium, such as fresh meat, is 4.8 million rads. It is possible that further work may change this value, and it is probable that the value will vary, depending upon other pertinent factors which

may be present. For example, it may be anticipated that a sufficient amount of salt present will lessen the amount of radiation required. It has been determined, also, that a radiation treatment of the order of 1 million rads increases the heat sensitivity of this organism so that the combination of radiation and heat might be effective in obtaining sterility with lower requirements for each through a synergistic action.

Regardless of what figure is finally settled on as the sterilization requirement for meats, one can with reasonable certainty state at the present time that a substantial amount of radiation will be needed. This is significant in that ionizing radiation induces certain changes in meats which affect their sensory properties. The most notable of these, as is well known, is a change in flavor. It has been found that the degree to which this foreign flavor develops varies in proportion not only with the amount of radiation used but also with the species from which the meat is obtained. Beef apparently is most sensitive to this change; pork is a great deal less sensitive.

The chemistry of this sensory change is not known. Work at the American Meat Institute Foundation has suggested that its origin may lie in sulfur-containing proteins. That it is associated chiefly with protein constituents seems to be rather well established. Some part of the sensory flavor change undoubtedly is associated with fat, since it is possible to detect the presence of carbonyls in fat after irradiation. There is also evidence of other compounds which might be contributing to off-flavors.

There is some disagreement as to the significance of the flavor changes which have been observed. In general, I believe that it is fair to say that most people dislike the radiation-induced off-flavors. Some reasonable arguments exist about products which show relatively low levels of off-flavors, the question being whether such low levels will impair acceptability for consumers. Thus, for example, there are those who hold the view that a number of pork products can be prepared which are acceptable, at least for certain uses.

In addition to flavor changes, there are other sensory changes which have been observed. Most notable of these have been changes in appearance. There can be degradation of fresh-meat color to a brown, and, strangely, there can be an imparting of pink color to cooked meats. It has also been observed that radiation in the amount of approximately a few million rads can cause a tenderization of beef.

A major area of concern for sterilized meats is the inactivation of the enzymes. It has been shown that very large amounts of radiation are required to produce complete destruction of spoilage micro-organisms. It is generally conceded that radiation cannot be used to produce enzyme inactivation because of the large quantities of radiation needed. Storage tests on meats irradiated with quantities sufficient to destroy only the micro-organisms have shown evidence of enzyme activity in the production of off-flavors and in the growth of crystals of the amino acid, tyrosine, on the surface of meat. The present thinking is that heat will be required to inactivate the enzymes in meat, and this leads immediately to the conclusion that sterilized meats probably will be cooked meats.

The maintenance of sterility assumes that the meat will be packaged in a tight container which will prevent the re-entry of additional spoilage micro-organisms. While there may be some differences of opinion on this point, it would appear that the only satisfactory containers available today are the kinds used for thermally processed meats, namely, a metal can or a glass jar. Plastic films show defects in a number of areas. They may not be sufficiently impermeable to oxygen, they may be difficult to seal, and they undergo chemical changes when irradiated which affect their functional properties or which impart flavors to the foods.

As with sterilized foods, one of the most important pieces of information for pasteurized products is the dose requirement. This appears to be better

known, although it is necessary to specify the intended use and life of the product in order to be able to specify precisely what dosage is required. It appears that approximately 100,000 rads is a useful dose on fresh meats. Bacterial growth will be sufficiently impeded so that, at the end of 2 or 3 weeks, microbial spoilage has not occurred. This amount of radiation is sufficiently small as to impart little or no off-flavor. Consequently, off-flavor problems with pasteurized meats are at a minimum. However, the objective is to obtain a product stability with respect to all its desirable properties. It has been our finding that discolorations, in particular, occur at refrigerator temperatures in a matter of a few days, a fact which so limits the life of the product from a salability standpoint that such pasteurized fresh meats have little commercial significance. It may be that, by using radiation in combination with other treatments, the desirable properties of these meats can be maintained for a sufficiently long period so that utility exists. One might hold the view that it is only by looking in such directions that one could expect to justify the use of radiation in preparing pasteurized meats.

Early in the consideration of the use of radiation for the preservation of foods, questions were raised on the effect of the radiation on the wholesomeness of the product so treated. Since those early days a great deal of work has been done to determine whether radiation induces undesirable changes. Nutritional changes are of the same order of magnitude as those experienced in thermal processing. There has been no demonstrated evidence of toxic material attributable to the radiation. Wholesomeness studies under way have included not only the usual criteria for wholesomeness but, in addition, others directed toward many specific points, including carcinogenic activity. While investigations on the point of carcinogenicity are incomplete at present, it can be said that the record for irradiated foods is clear so far. Finalization on this point and others, however, must await completion of the tests in progress.

One cannot have a discussion on irradiation without some analysis of the economics of the process. It is possible today to calculate costs for treatment of foods with electron beams. Machines for generating these beams exist and can be purchased at known prices; their operating characteristics and costs are known.

The costs can be expressed conveniently in terms of a million rad pounds (megarad) units. Costs such as are available, however, must be considered as estimates, since actual commercial experience is lacking. Further, generator costs are based on today's prices, which may be high because of low volume of production of these units. In arriving at costs, the assumption is necessary that efficient utilization of the equipment is possible; that is, that the process allows this, and the volume of product available to be treated permits a high use factor. With such considerations one arrives at costs as low as about a 0.25 cent per megarad pound and as high as 10 cents per megarad pound, depending on the electron generator chosen. At this time, then, the statement "from a fraction to a few cents per pound" is our best estimate. Considerable detailing of the particular process and of the equipment for it plus actual experience are needed to define costs more closely.

Attempts have been made to project current charges for isotopes such as cobalt-60 or cesium-137 to their ultimate costs, assuming large-scale production. If one accepts such projections, it appears that costs for cobalt-60 will be substantially higher than exist today for electron generators. In the case of cesium-137, projections indicate the possibility of costs competitive with some electron generators.

The commercial use as sources of gamma radiation of fuel elements during their "cooling" period will in part depend on the availability of sufficient of these in steady supply, which in turn will be governed by other uses for reactors, probably the generation of electric power. The utilization of reactors designed specifically for food irradiation, either in whole or part, awaits tech-

nological advances as well as economic justification.

It has been proposed that gamma radiation be used to control trichinosis originating with pork. This proposal is based on findings that relatively small amounts of radiation (20,000-30,000 rad) will break the life-cycle of the parasite by interfering with the maturation and reproductive processes. The specific proposal is to treat entire hog carcasses so that all pork, regardless of further treatment, will be safe for consumption with respect to trichinosis.

This is a very involved subject. A full discussion would include consideration of feasibility, from a practical viewpoint, of desirability from public health consideration, of economics, and of other related factors which have a bearing on trichinosis. Let us simply state that the conclusion can be drawn that irradiation is not a practical approach at the moment. In the future, if certain necessary developments occur in the radiation field, especially in the area of economic sources, irradiation might become an approach.

There is, however, a fortuitous and particularly pertinent concomitant event which offers another and very simple approach to the breaking of the life-cycle of Trichinella spiralis in swine. It is generally agreed that the major source of infestation of hogs has been raw garbage containing raw pork fed to them. This practice of feeding garbage has been widespread in the United States, and until recently no successful device to require the cooking of such garbage was available. The recent outbreak of vesicular exanthema, a disease having significant economic considerations for swine producers, has led to the situation that there now are laws in forty-seven states requiring cooking of garbage before feeding. According to a USDA report, as of December, 1957, 95.2 per cent of swine fed garbage were fed cooked garbage. Hence one may anticipate a marked reduction of the incidence of trichinae-infested hogs and no need, therefore, to give further consideration to the use of radiation for treatment of pork in connection with trichinosis.

What might be said of the future for irradiation? The future is certainly full of problems. Control of the sensory changes in meats is essential if we are to provide the civilian market with products reasonably similar to those now supplied. The sterilization requirements must be defined. The enzyme problem must be solved. Wholesomeness must be established. Useful applications of radiation must be developed. Products must have properties not now obtainable by any other procedures, or costs must be lower than competitive processes. Adequate sources of radiation must be available. Problems of packaging must be solved. Control of the process through suitable radiation dosimetry must be secured. While much has been done to date, problems exist in all these areas. Until at least the major ones are solved, it does not seem reasonable that radiation can be utilized commercially. One is therefore led to the conclusion that radiation as a food-preservation process for use in civilian foods still lies in the future.

III

FRESH-MEAT CHARACTERISTICS WHICH INFLUENCE PACKAGING REQUIREMENTS

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If one were to summarize in one word the fresh-meat characteristic which influences packaging requirements, it would be "color." The consumer has been educated to desire meat in the cherry-red form of oxymyoglobin and to reject meat which has the brown color of metmyoglobin. What factors influence the color of meat? In broad terms we can classify four parameters which influence color: surface dehydration, temperature, oxygen requirements of the meat, and bacterial contamination. Little will be said here on dehydration except that in small degrees it may be helpful, but certainly it is deleterious when it occurs to any great extent. In the first several days of packaging, bacterial contamination is not the limiting factor on meat which has been cut and packaged under proper conditions of sanitation. Therefore, this discussion will be limited to the effects of temperature and oxygen requirements.

Briefly, let us consider the chemistry of the conversion of oxymyoglobin to metmyoglobin:

$$\text{Oxymyoglobin} \rightleftharpoons \text{Myoglobin} + O_2 + 1.5\ O_2 \rightleftharpoons \text{Metmyoglobin.}$$

According to George and Stratmann (1), the reaction proceeds by a dissociation of oxymyoglobin into oxygen and myoglobin requiring an additional 1.5 moles of oxygen to form metmyoglobin. These authors also studied the kinetics of this reaction and established a temperature coefficient of 2.8 per 10°C. change. This is a very important point to keep in mind, since, by increasing the temperature by 10°C., we cause an almost threefold increase in the amount of metmyoglobin being formed.

These investigators also established the relationship between the reaction rate of metmyoglobin formation and the partial pressure of oxygen. At 30°C. they found that increasing the partial pressure of oxygen above 30 mm. of mercury had no effect on the reaction rate. However, lowering the partial pressure below 30 mm. caused an increase in reaction rate, until the pressure was decreased to 1.5 mm. of mercury, at which point a maximum reaction rate was observed. Below 1.5 mm. the reaction rate decreased to zero as the partial pressure of oxygen became zero.

At this point we might well consider how and why a fresh-cut meat surface absorbs oxygen. Before being cut, the interior of meat is essentially devoid of oxygen, as indicated by the purple color of myoglobin and the absence of metmyoglobin. On cutting, the meat will tend to approach its equilibrium conditions with respect to the partial pressure of oxygen in air and its own temperature. In other words, the myoglobin becomes oxygenated to form oxymyoglobin, and the water in the meat will absorb oxygen to achieve saturation. Oxygen is also being utilized for the enzymatic oxidations and, to a minor extent, the autoxidation of fats and myoglobin.

The surviving respiratory enzyme systems have been studied by Andrews

et al. (2) and by Grant (3), both studies finding the succinic dehydrogenase system as the major surviving oxidative enzyme system in meat. The optimum pH for muscle succinic dehydrogenase is approximately pH 7.8. In Figure 1 we can observe the effect of pH at three different temperatures, 30°C., 20°C., and

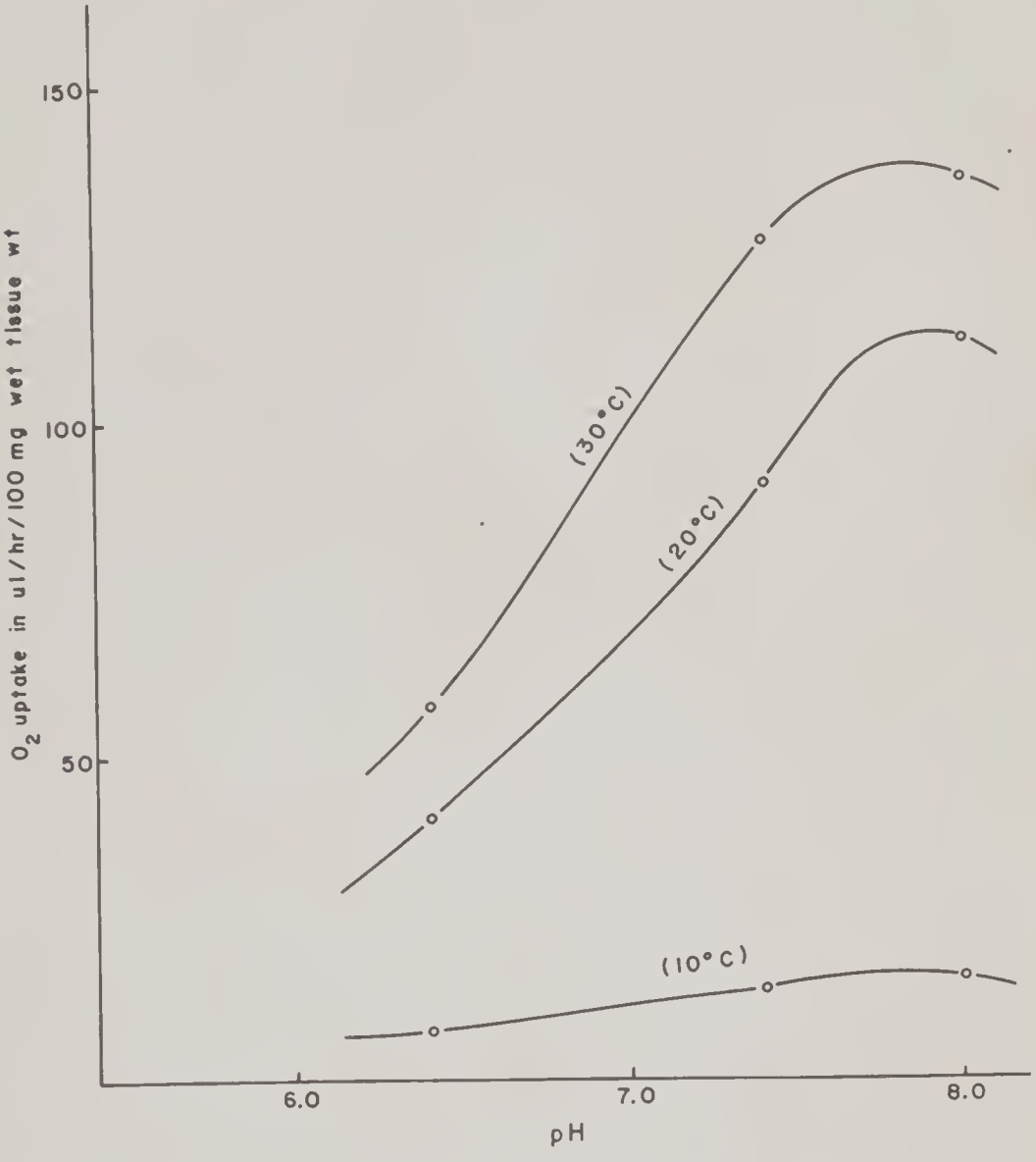


Figure 1. The effect of pH on the oxygen uptake of muscle succinic dehydrogenase at three different temperatures.

10°C., for the succinic dehydrogenase system. It should be noted that, as the pH decreases, the rate of oxygen uptake decreases, with a tendency for the curves to converge at lower pH values. In Figure 2 the rate of oxygen uptake is plotted against temperature at pH values of 8.0, 7.4, and 6.4. Again, we note the convergence of the pH curves as temperature is decreased, so that for low temperature the pH effect on enzymatic oxygen uptake is minimized. From these data it becomes apparent that a decrease in temperature is our best means of holding the enzymatic demands for oxygen to a minimum.

The solubility of oxygen in water increases as the temperature decreases. In Figure 3 the solubility of oxygen in microliters per gram of meat is plotted against temperature for 158 mm. partial pressure of oxygen or air. The interest here is only in that portion of the curve below 10°C., which for all practical purposes is linear. If one consults Figure 2, which is a plot of enzymatic activity versus temperature, it will be seen that at 10°C. enzymatic activity is

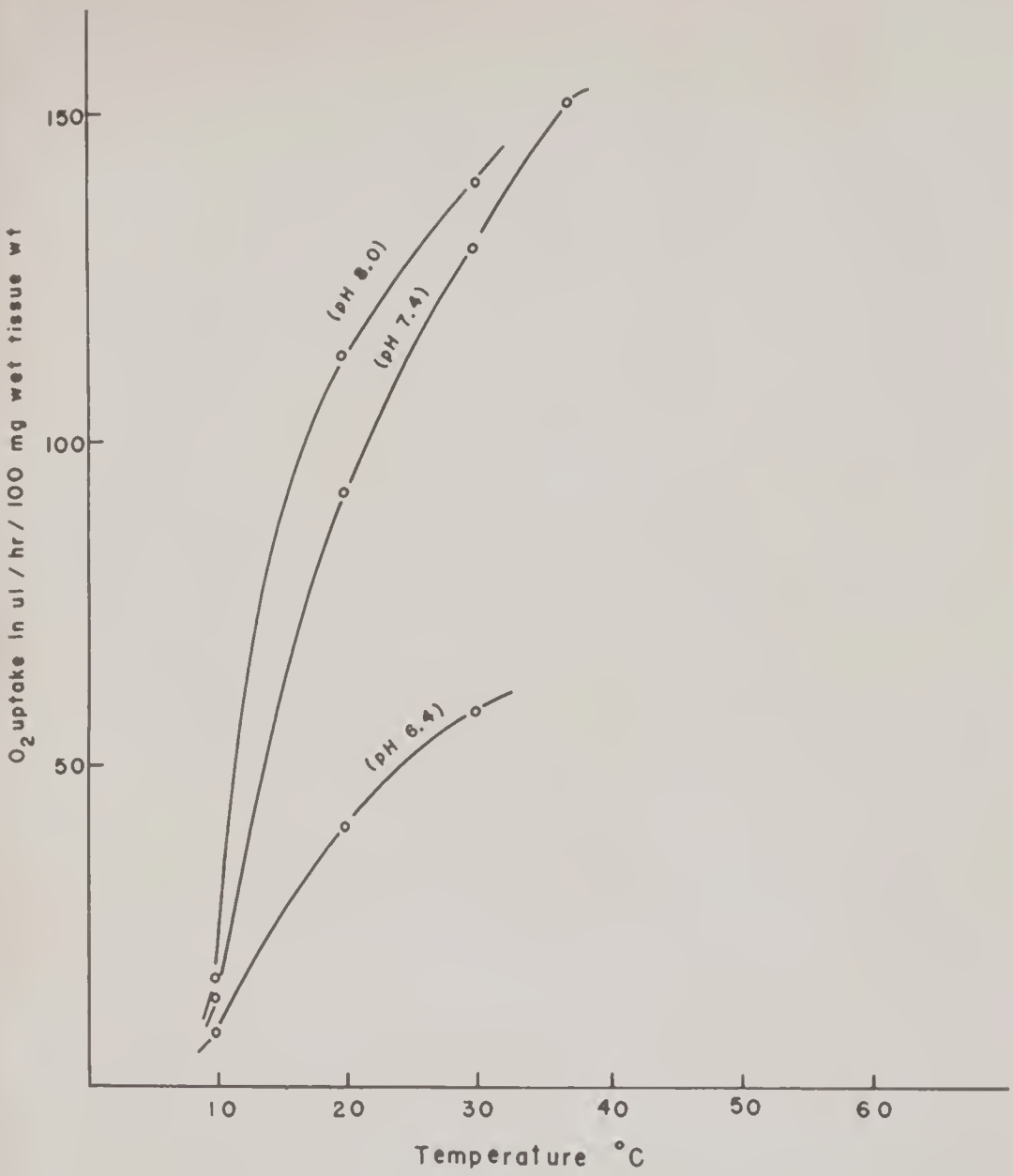


Figure 2. The effect of temperature on the oxygen uptake of muscle succinic dehydrogenase at three different pH values.

already decreased considerably. Thus, below 10°C., oxygen penetration into meat varies inversely with temperature or directly with oxygen solubility. The depth of penetration of oxygen for any particular temperature can be readily checked by the formation of a metmyoglobin ring in the interior of the meat. Figure 4 illustrates that the formation of a metmyoglobin ring will vary with temperature, the ring being established deeper for meat held at lower temperatures. This ring is formed at the farthest point of oxygen penetration, where oxygen tension is low and metmyoglobin formation is at a maximum. The increase in penetration naturally means an increase in the number of grams of meat which will be exposed to oxygen for any given surface. For example, if we consider a 10-sq. cm. surface of meat, a penetration of 1 mm. is approximately equal to 1 gm. of meat being exposed to oxygen, while 2 mm. would be equivalent to 2 gm., and so forth, as penetration becomes deeper.

The oxygenation of myoglobin proceeds on a mole-for-mole basis. Ginger et al. (4) have found an average of 4 mg. of myoglobin per gram of fresh tissue in beef. If calculated in terms of oxygen, it is found that approximately 5 μl. of oxygen are required per gram of tissue to oxygenate the myoglobin. Since penetration of oxygen increases as temperature decreases, a decrease in temper-

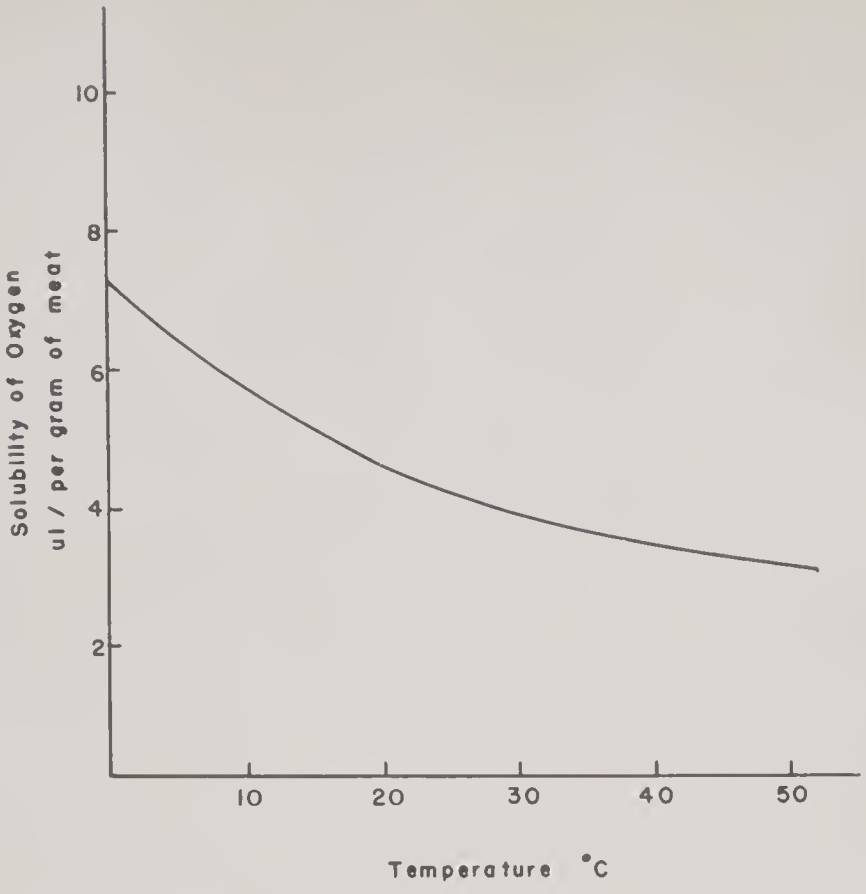


Figure 3. Oxygen solubility in meat at various temperatures in an air atmosphere.

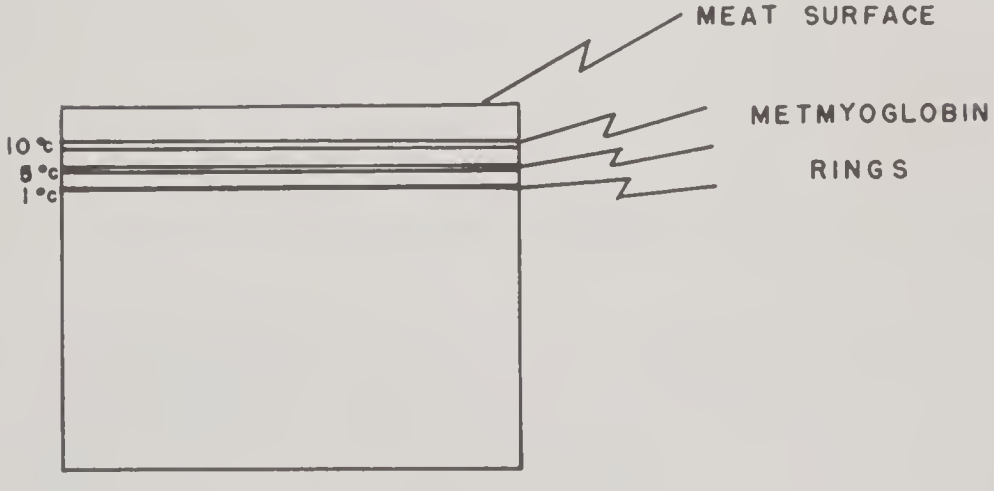
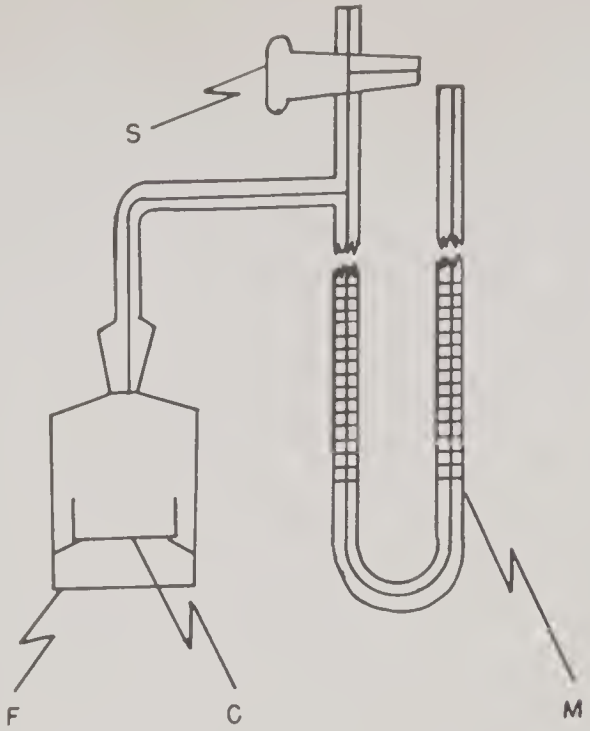


Figure 4. Metmyoglobin-ring formation as seen on a vertical cut of meat.

ature causes an increase in the number of grams of meat exposed to oxygen and the total amount of myoglobin oxygenated.

In our laboratories the measurement of oxygen uptake of fresh beef muscle has been made, using a Warburg apparatus with oversized 100-ml. flasks. Figure 5 shows the manometer, flask, and meat sample cups used for these experiments. The surface area of the sample is approximately 11.9-sq. cm. The sample cup is a 50-ml. beaker which has been cut down to a depth of 18 mm. Circular samples, 38 mm. in diameter and 36 mm. thick, were cut from either the semimembranosus or the adductor muscles of round steak. One sam-



S - Stopcock
F - Warburg Flask
C - Sample Cup
M - Manometer

Figure 5. Apparatus for measurement of oxygen uptake.

ple cup was placed on each end of the steak sample and cut through the middle to provide two identical surfaces. Each experiment contained sixteen samples, or eight pairs of identical surfaces. Standard manometric procedures could not be followed, since it was necessary to measure uptake of oxygen as soon as the samples were cut, thus allowing no time for equilibration of the flasks. To minimize temperature effects, all experiments were performed in cold rooms having temperatures approximating the water-bath temperature. Manometer readings were taken at half-hour intervals for the first few hours and at longer intervals thereafter. Five milliliters of 5 per cent potassium hydroxide were placed in the bottom of the reaction flasks to absorb carbon dioxide, except in those experiments in which carbon dioxide was being measured. Oxygen uptake experiments were studied at 30°C. and 1°C.

At 30°C., oxygen uptake was about 75 μl. per gram, largely due to enzymatic demand for oxygen, since oxygen penetration into the meat was only 2 mm. At 1°C., penetration of oxygen was about 14 mm. into the meat. In Figure 6 the oxygen uptake at 1°C. has been plotted against time. The uptake of oxygen is linear with time up to 15 hours, at which time the slope of the curve decreases. Penetration of oxygen into meat occurs at about 1 mm. per hour, so that at 15 hours the meat has come to equilibrium with the atmosphere in respect to the myoglobin being oxygenated and the moisture achieving saturation of oxygen. The uptake of oxygen at this point is due to enzymatic demands alone. To show that this is a reasonable assumption, we can correct the lower portion of the curve by subtracting the uptake of oxygen required for oxygenation and saturation and achieve the same slope which has been found for oxy-

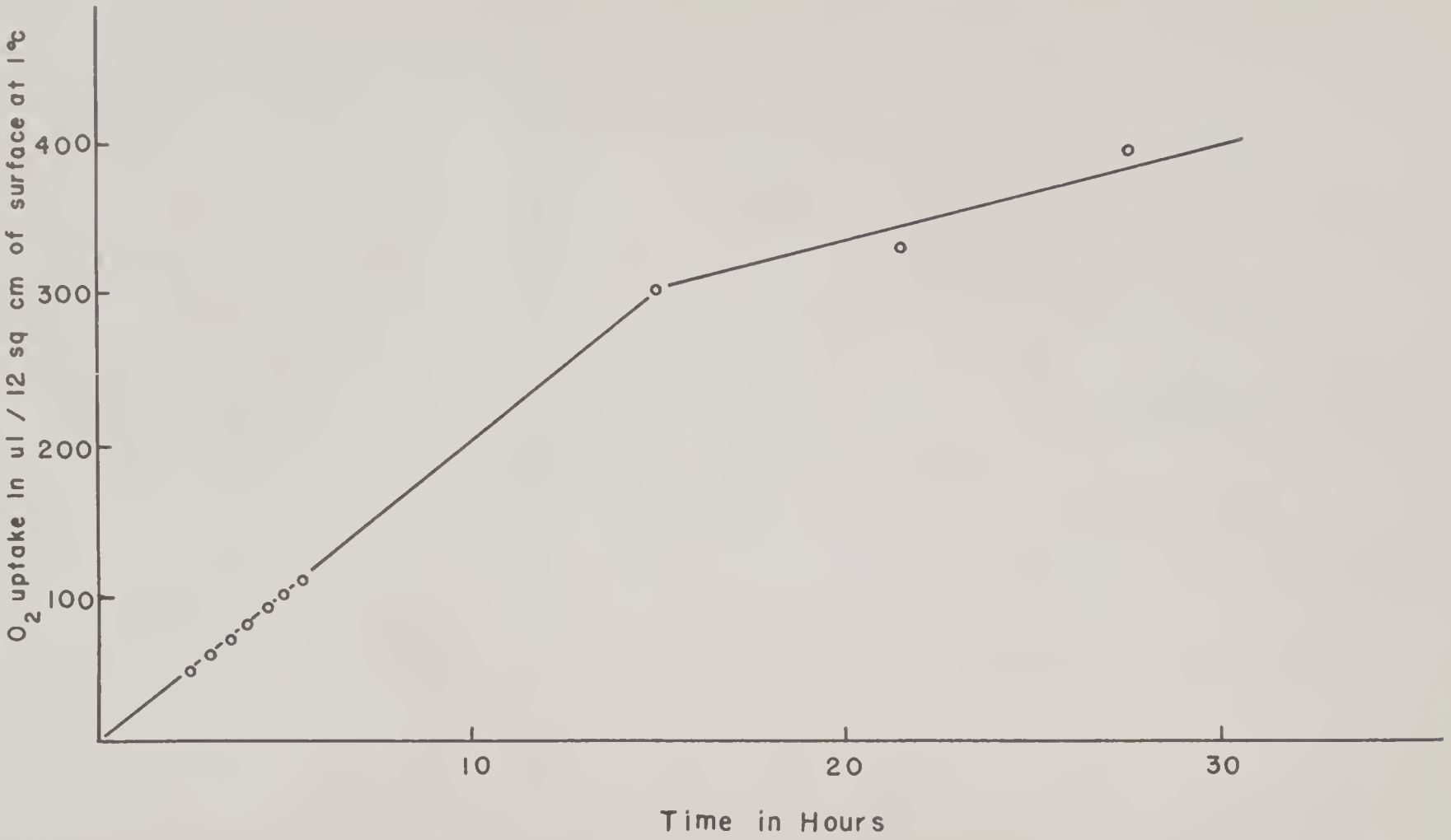


Figure 6. Oxygen uptake of a fresh-cut meat surface.

gen uptake after 15 hours.

An important factor in effecting accurate oxygen-uptake measurements is the evolution of carbon dioxide. In a fresh-cut meat surface, carbon dioxide evolution is due largely to the reversal of the carbon dioxide to bicarbonate ratio as calculated from the Henderson-Hasselbalch equation. The reversal in ratio is caused by the drop in pH from 7.2 to 5.8, which occurs during post mortem changes. In Figure 7 the evolution of carbon dioxide is plotted against time. In

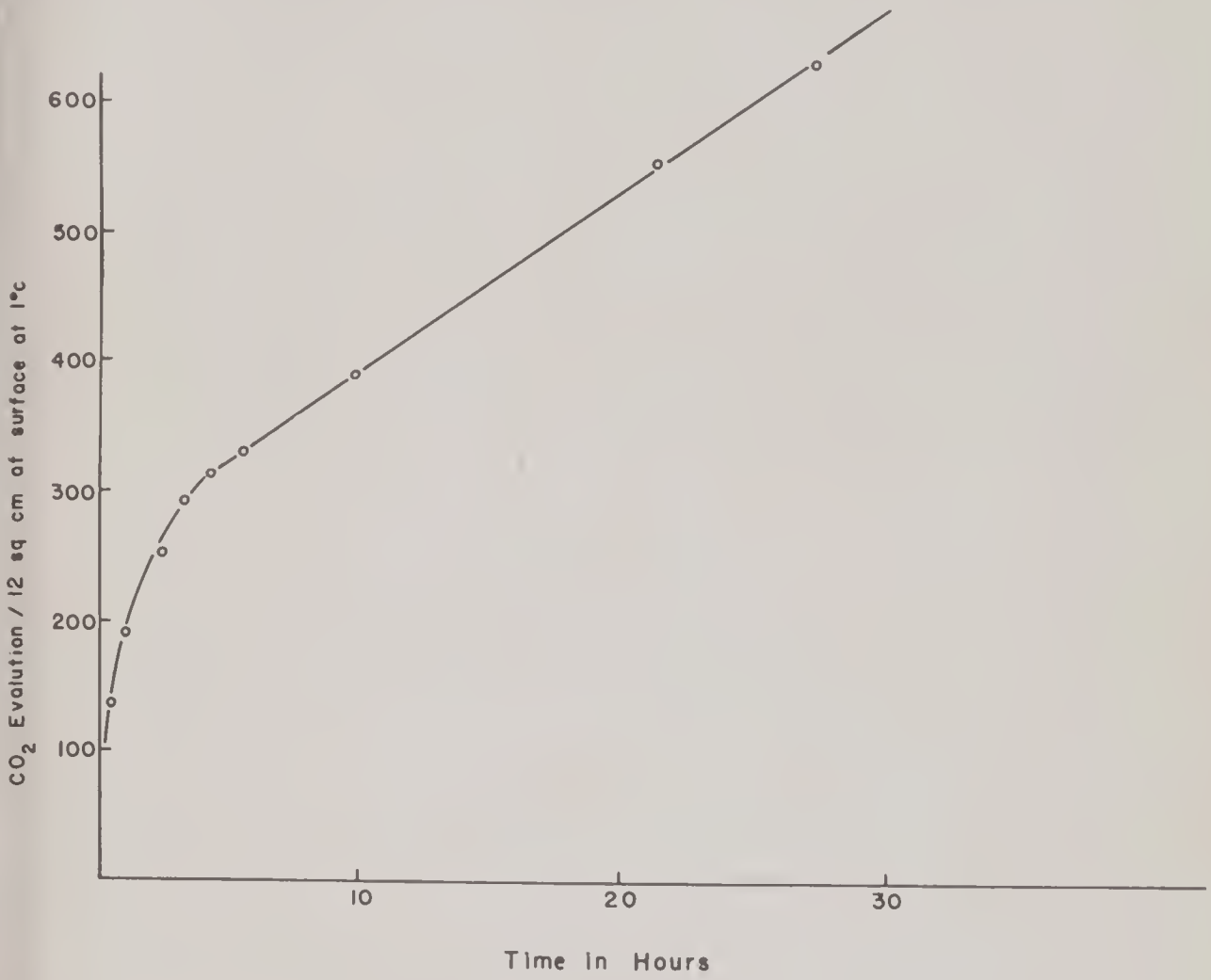


Figure 7. Microliters of CO_2 evolved from a fresh-cut meat surface.

the first 3 hours there is a large evolution of carbon dioxide that involves mostly the surface and causes a compression of the meat surface. In the measurement of oxygen uptake the flasks contain potassium hydroxide to absorb the carbon dioxide produced. Carbon dioxide which is absorbed from a solution causes a compression or loss in liquid volume. After 3 hours the evolution of carbon dioxide from a meat surface becomes linear, since compression effects no longer affect the sample. It is necessary to correct the oxygen-uptake curves for the apparent uptake in the first 3 hours, owing to the decrease in volume of the sample. Figure 6 was corrected for this volume compression before the oxygen uptake was plotted.

An attempt was made to determine what effect the addition of either substrates or inhibitors of the oxidative enzymes would have on oxygen uptake when added to the surface of meat samples. The addition of 1 ml. of a 1, 2, or 5 per cent solution of sodium succinate to the surface of meat caused no change in oxygen uptake when compared to a sample to which no substrate had been added. The addition of inhibitors such as sodium malonate or zinc sulfate had no effect on the oxygen uptake when added in the manner described for substrate

addition. The main reason for failure of inhibitors or substrate to influence oxygen uptake was probably due to poor diffusion of these substances into the meat sample. If these substances diffuse only 1 mm. into the sample, they would be influencing only one-fourteenth of the total sample, since total penetration is 14 mm., which is certainly too small a change to measure manometrically at low uptake levels.

The conclusion derived from this work and that of George and Stratmann (1) is simply that it is advantageous to cut and package meat at 0°C. It is not necessary that the packaging room be held this low, but the meat temperature always should be kept low. In packaging, the meat should be held in the packaging room for as short a time as possible. Any period of time in which the meat is cut and left unpackaged in air or in higher oxygen atmospheres at low temperature will be beneficial to color preservation. The optimum to be approached at these low temperatures is when all oxygen uptake is due to enzymatic demands, that is, the requirement for solution saturation and oxygenation of myoglobin have been met. The use of enzyme inhibitors appears to be a fruitless approach, since their penetration into meat is not great enough to have a substantial influence on total enzymatic uptake. The maintenance of proper sanitation conditions in cutting and prepackaging is always a necessary requisite in good packaging standards for meat. Otherwise, complications not considered in this discussion will be encountered.

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IV

PACKAGING TO MEET CONSUMER DEMANDS

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Independent Grocers Alliance
Chicago, Illinois

Inasmuch as I am the only retailer in the room and in such distinguished company, I am almost as nervous as the young minister who was to give his first sermon. He had been trained under an older minister, who knew the young man's problems from past experience. So he said to the young minister, "I know how nervous you are, and I have placed a glass of gin on the pulpit, and, as it looks like water, the congregation will not know the difference. So, if you feel a little nervous, just take a sip." The young minister approached the pulpit and with trembling fingers grabbed the glass and took a big slug—and started his sermon. Still a little nervous, he continued to sip from the glass. After about the fifth sip, he became quite eloquent and finished his sermon in a burst of oratory. As he walked over to the old minister, he asked, "How did I do?"

The old minister said, "Son, you done fine, but, may I remind you, David slew Goliath—he did not stomp the living hell out of him."

I will probably remain a little nervous—and I want to do a little stomping, in a most friendly way—because I believe that a friend is a fellow who tells you what you ought to hear rather than a fellow who tells you what you want to hear. My first friendly criticism is the surprises in the packaging field that the industry pulls on us by its ever changing designs and ideas in prepackaging. I am cognizant of the fact that secrecy of the new product or design is to gain a competitive advantage; but, if we, as retailers, are not prepared, we must either fight the program or go along for fear it might prove to be a winner.

I do not believe that you want our mere acquiescence. I think that you want our enthusiastic support.

The industry sprang such a surprise on us in introducing a packaged frozen-meat line. We were totally unprepared to cope with the case space in our retail stores or with the equipment for storage in our back rooms. Frankly, we were afraid to say "No." So we went along. Why not—maybe this was it.

Therefore, my first suggestion is that, if you cannot trust all of us to keep your plans in confidence—and I do not think you can—surely there are enough honorable retailers so that you could have some kind of a consulting committee of retailers with which to discuss the problems and potentialities of your future plans.

Personally, I view with suspicion all secret plans, because, if you have a good sound idea, it came from months or years of thought before it even arrived at the production stage. If the idea has merit, once the product is launched, imitation is almost immediate.

If we want to talk about packaging for consumer demand, let us talk about the Wallett Pack Lunch Meat. It was sprung on us again as a surprise, but, when this extra cost to the product was added, the industry violated the retailer's creed of bringing the finest food to the consumer at the lowest possible cost. The addition of the carton over and above the plain Saran wrap meant only two things to us, even though our suspicions may have been unfounded: that you have added to the cost of the package or that you had taken enough quality out of the product to pay for the added cost. We again had to ask our-

selves this question: Is the industry dedicated to the task of bringing the best food to the consumer at the lowest possible cost—or is it simply seeking a competitive advantage? So, monkey see, monkey do. Many in the industry changed to the Wallett Pack. So again we went along—maybe this time you have it—but again you do not get our enthusiastic support. You again get our acquiescence, and at the present we see no particular future for the Wallett Pack.

Now, let us take an item we all recognize—bologna. Because the industry packs and designs so many of the luncheon meats on a 6- or 8-ounce basis, they know that the 6- or 8-ounce package of bologna fits your machine or production line; so you pack bologna in the same pattern. What you apparently do not know is that the apartment-house dwellers are not bologna tonnage customers. They buy some bologna, also the luxury lines—pickle and pimento, barbecued ham, pickled tongue, etc. Bologna is not purchased just by poor people. It is a favorite meat for many of our rural friends. They have the advantage of eating beef, pork, veal, and lamb, fried, roasted, and boiler—in many cases killed and dressed by themselves. They like bologna because it has a flavor they cannot reproduce. Picture, if you will, a farmer with five children, who, after plowing his 20 acres, comes in to lunch to find a 6- or 8-ounce package of bologna for a noon snack. He wants and deserves a 1-, 1-1/2-, or 2-pound package bought as cheaply as he can, and he wants you to design and produce the best package at the lowest possible price.

So, if I am to stick to my subject of packaging to meet consumer demands, then I offer for your consideration the fact that you must know an awful lot about the consumer and, if you want to know a lot about the consumer, that the best place I know to learn something about her is at the retail level.

We can discuss modern production methods, scientific packaging, and color dynamics in our ivory towers, but, when it comes down to the retail sale, which is the basis of the industry's total tonnage, dependable quality must be our first consideration—without this ingredient no package, regardless of design, can survive.

Let me illustrate. Just a few years ago, in a small town in Illinois, an individual started making a whole-hog, 1-pound pork-sausage roll. In a matter of a few years his sales per store, in that area, are greater than the combined sales of all other packer brands, notwithstanding the fact that the cost of his product is 12 to 15 cents higher than the retail of all other brands. The point is: it is doubtful if the cost of his artwork, design, and engravings for his brand cost a fraction of the cost of the major packers. But he started with a basic quality, which is the first basic step in good packaging.

Too often, because of apparently excellent design, coupled with strong promotion and price, the survey of a test market does not present a true picture of what may be expected in future repeat business.

Therefore, in prepackaging for the consumer, you must know how the consumer shops in a food store and what motivates her.

The three strongest tools in retail merchandising and in their proper order are: (1) display, (2) reputation, and (3) price. With display and price, you can induce anyone to try any product once, but, if the quality of the product does not come up to her expectations, you will fail to build a reputation. And, without reputation, repeat sales cannot be maintained.

Therefore, by simple trial and error, the customer soon learns to trust a brand in the grocery department and becomes a repeat customer. In the fresh fruits and vegetable department she trusts herself. She squeezes her own tomatoes, pinches her own peaches, and, if you do not watch her, she will even trim her own lettuce.

In the meat department, however, she trusts the retailer or the packer brand, and, when that trust is betrayed, she shops around until she finds the store she can trust. We rarely lose a customer because of our groceries. It

is almost always because of our meats or produce. The retail industry has spent thousands of dollars trying to teach the housewife how to judge meat, but she refuses to learn. She insists on trusting some retailer or packer brand, and, even then, the packer's brand or package is at the mercy of the individual retail operator because, if he neglects to maintain proper rotation of the packer's brand and sells stale product, the customer seeks another retailer or another brand, nullifying all your fine work. So, you see, you really have a retail problem that should be a part of your consideration when prepackaging is considered.

If the packer splits fresh butts—and some do—for the purpose of manufacturing smoked butts, no matter how it is packaged, one customer is going to get a lean butt and the other customer is going to get one with too much fat in it. She does not know the reason for it, but she will resist the package.

Another example of prepackaging that retards sales is the foil-pack dried beef. This type of package presents a problem to both the customer and the retailer. She cannot see the product, and we cannot check the freshness of the product. On tests we made in two midwestern cities, the visible package outsold the non-visible package fifty to one at the same retail.

Another example is the flip-top 1-pound bacon package. Theoretically, it was the answer to keep bacon from losing its color, because it excluded light. However, it retarded sales, simply because the consumer still buys meat with her eyes, and it seems to be a fact that every package that diminishes visibility of the product has an adverse effect on sales.

Therefore, as we see it at the retail level, as we develop more prepackaging for the consumer, our considerations must be:

1. Scrupulous honesty of quality.
2. Let the consumer see as much of the product as possible.
3. Identify the product properly.
4. Do not change the appearance of the product too fast. Consumers resist sudden changes.
5. With fresh or frozen red meats, do not overtrim. Give the product a good trim, but overtrimming simply puts the product out of line price-wise.
6. Learn all you can about the shopping habits of Mrs. Consumer. She is a more complex person than you think. For example, in a neighborhood that offers only service meats, a door-to-door survey might show that 50 per cent of the housewives do not want self-service meats. If the market is properly converted, however, only 3 per cent of these same customers will resist self-service meats.

I am sure that you will continue to bring to us important advances in packaging techniques because you are not thinking like one packer I called on a few years ago. As he was showing me through the plant, we came to the prepackaging room, and he pointed out the foreman to me and said, "See that fellow? When he first took hold of this department, we were only producing 10,000 pounds of product per week."

I said, "Is that so? What are you producing now?"

He replied, "Ten thousand pounds." But it goes to show how easy it is to hold your own in our organization.

In closing, I want to assure you that we at the retail level know we need all your talent. We know we are dependent on you folks for the necessary research for the advancement of our future know-how.

We hope, as you become better acquainted with us, you will find some small need for us retailers, too.

V

DIETARY FATS, BLOOD FATS, AND ATHEROSCLEROSIS

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When someone who has devoted a good portion of the last eight years to study of effects of vegetable fats addresses an audience made up of people who are primarily concerned with the meat industry, he has a pretty clear idea how Daniel felt when he peered into the den of lions. Let me hasten to reassure you, however, by stating that at dinner last evening I thoroughly enjoyed my lamb chops.

In 1950, as part of a study designed to evaluate the effects of certain hormonal entities upon fatty-acid oxidation, we maintained a patient on a diet containing literally nothing but fats, except for essential vitamins and minerals. In the course of this study, among other things, it was noted that the level of plasma cholesterol and phospholipids fell to a remarkable degree and that this fall was maintained as long as the pure fat diet was continued (1, 2). Vegetable fat was used in this study because, being a liquid fat at room temperature, it was easier to emulsify than one which was solid at room temperature. Initially, we were inclined to believe that the decrease in the plasma lipids was referable to the effects of hormones, which were co-administered. Subsequent studies, however, indicated that, whenever appreciable amounts of a variety of vegetable fats, including soy, cottonseed, corn, and, more recently, safflower oil were administered (in the absence of any hormonal medication), this same decrease in plasma cholesterol, in phospholipids, and, usually, in neutral fats resulted.

These observations raised certain questions, among which were: Was the decrease due to the absence of something, with particular reference to cholesterol in the vegetable fat, as compared to animal fat? Was the change due to the presence of something? Substitution of animal fat for the vegetable fat resulted in a prompt rise in the plasma lipids. However, when very large amounts of cholesterol were added to the vegetable fat, little or no rise in plasma lipids was noted. It therefore seemed reasonable to conclude that the change was not referable to the mere absence of cholesterol in the vegetable fat (1).

Vegetable fats contain sterols and a variety of phospholipids. Studies were carried out in which large amounts of vegetable sterols and large amounts of vegetable phospholipids were added to the diet under controlled conditions. In some instances small decreases in cholesterol were noted when vegetable sterols were added, but these changes were unpredictable and usually of small magnitude (3, 4).

In the case of the vegetable phospholipids, a few preparations did cause a rather marked decrease in the plasma lipids, but here, too, the results were inconstant (3, 4). Later studies have shown that the phospholipid preparations which were associated with the decrease in plasma lipids were those which contained relatively large amounts of unsaturated fatty acids.

By exclusion, therefore, it seemed rather probable that the plasma-lipid-lowering effect was referable to the type of fatty acids present characteristically in the vegetable fats which had been used, namely—the polyunsaturated fatty acids. It may be well to note here also that the level of plasma lipids after

inclusion in the diet of significant amounts of vegetable fat (i.e., more than 60 gm.) was much lower than when a diet was administered containing no fat whatever.

Studies carried out during the past year using highly purified preparations of linoleic acid, usually as the ethyl esters but in some instances as the triglycerides, have shown the same decrease in plasma lipids that is obtained when one administers natural fats containing equal or greater amounts of linoleic acid. In the case of similar oleic acid preparations, the plasma lipid levels have been about those noted with the fat-free diets or, on occasion, slightly below. In no instance has the lipid lowering been of the magnitude obtained with linoleic acid preparations (5).

Purified preparations of arachidonic acid have not yet become available. One study carried out with a crude liver phosphatide preparation containing 12.5 per cent arachidonic acid was associated with a very marked decrease in the level of plasma cholesterol and phospholipids (5). The magnitude of this decrease was greater in this patient than with larger amounts of purified linoleic acid preparations. It is impossible to state at this time whether the effect was due to the arachidonic acid per se or to some other properties of this particular phospholipid preparation.

If, then, fats rich in linoleic acid are capable of producing the changes noted in the plasma lipids, is this a desirable effect, with particular reference to atherogenesis? If the answer is in the affirmative, does this mean that one must exclude all saturated fats (animal fats) from the diet? In the case of the human, there is still no totally unequivocal evidence to indicate that lowering of plasma lipids exerts a favorable effect on the formation of atherosclerotic plaques or upon the dissolution of existing plaques. Recent studies in our laboratory in patients with intermittent claudication (atherosclerosis involving the arteries of the lower extremities) have shown rather marked improvement in many such patients during the course of intake of diets high in linoleic acid. If the results of such studies continue to move in the same direction, there will be increasing reason to believe that linoleic acid may exert a desirable effect on existing atherosclerosis and, presumably, a prophylactic effect insofar as atherogenesis is concerned. A recent in vitro study by Rutstein is consistent with this philosophy (6).

With regard to the need for a diet which would contain only highly unsaturated fats, fortunately the answer appears to be in the negative. If one administers to normal and abnormal individuals diets containing large amounts of animal fat and then progressively replaces a portion of the animal fat with highly unsaturated vegetable fat, usually by the time 50 per cent of the animal fat has been so replaced, the plasma lipids fall to "normal" levels. In some patients with advanced vascular disease, it may be necessary for a time to have only unsaturated fat sources in the diet. For this purpose, we have devised three groups of diets. In Group I all the fat is from highly unsaturated sources. It is our custom to place patients with known disease upon such diets until the cholesterol levels in the plasma have fallen to 180 or below, with a proportional fall in the other plasma lipids. When this level has been maintained for two months, the Group II diet is introduced. This diet contains fish and fowl but no meat or dairy fat (fish and fowl contain appreciable amounts of linoleic acid and other polyunsaturated fatty acids). When the cholesterol has again been maintained for a period of two or more months at levels below 180, we shift to the Group III diet, in which significant amounts of meat, dairy fats, and eggs are included.

In the young, normal individual, on the basis of data so far available, it would seem probable that, if 10 per cent of the calories are in the form of highly unsaturated fats, a majority of such individuals will maintain normal plasma lipid levels. In men in the age group between forty-five and fifty-five who have no clinical evidence of vascular disease (this is not the same thing as saying that they have no vascular disease), it may be necessary to double

the amount of unsaturated fats and to diminish saturated fats and carbohydrates proportionately. In men between the ages of forty-five and fifty-five who have evidence of vascular disease, it may be necessary to triple or more than triple the polyunsaturated fat source and to reduce the saturated fat and carbohydrate proportionately.

What does all the foregoing mean in terms of possible modifications in the average American dietary? It is obvious that, under any circumstances, prophylaxis is better than treatment. It would seem to us that the most reasonable approach to the problem is by way of removal from the diet of hydrogenated fat. Dr. Brown has estimated that hydrogenation removes from the individual American dietary the equivalent of 5 kg. of linoleic acid yearly. It is recognized that discontinuance of hydrogenation will present certain problems with regard to shortenings and spreads, but none of these problems is even remotely insurmountable. With such modification, one can consume very reasonable amounts of meat, dairy fats, and eggs and yet have a diet which will contain what we believe to be physiologic ratios of polyunsaturated fat, on the one hand, and saturated fats and carbohydrate, on the other. We suspect that the years immediately ahead will witness significant changes in this direction.

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VI

FAT METABOLISM AND ENERGETICS

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Dr. Kinsell's paper in this program has summarized for us some of the very important evidence which relates dietary fats and blood fats with the most serious public health problem in the United States today—atherosclerosis. It would indeed be gratifying if those of us who are concerned with the study of the fundamental enzymatic reactions by which fats are metabolized in the body could provide answers to some of the questions raised by Dr. Kinsell. Unfortunately, there are very serious deficiencies in our knowledge of the most elementary aspects of the metabolism of lipides in the living cell, and further basic research in this area is badly needed.

In recent years, however, perhaps in part because of the recognition of the interrelationship between atherosclerosis and lipide metabolism, there has been a considerable increase in interest in fat metabolism, and noteworthy advances have already been made. Indeed, elucidation of the process of fatty-acid oxidation and synthesis, which will be the subject of this discussion, must be regarded as one of the outstanding achievements of modern biochemistry. Many laboratories have contributed to the development of this subject, most notably those of Lipman, Ochoa, Lynen, Green, Barker, and Lehninger. No attempt will be made here to review the literature of the subject in detail, since several recent and complete reviews can be found in the literature (1-3).

ENERGETICS AND FATTY-ACID OXIDATION

It is of course well known to this audience that fats represent the principal form in which food is stored in the animal body. The advantage to the animal of storing food reserves as fat is related to the fact that, gram for gram, the oxidation of fats yields considerably more energy than the oxidation of protein or carbohydrate. The complete oxidation of palmitic acid, a typical fatty acid, to carbon dioxide and water proceeds according to the following equation:

$$C_{16}H_{32}O_2 + 23\ O_2 \longrightarrow 16\ H_2O + 16\ CO_2. \qquad (1)$$

This reaction proceeds with the release of approximately 2,300,000 calories of free energy. If all this energy were to be released as heat, this process would be of little value to the living cell. But we now know that the over-all reaction described in equation (1) actually proceeds in a number of discrete, controlled steps so that the free energy which is released is made available to the cell in a chemical form in which it can be used for biosynthesis, growth, and function. Specifically, the energy which is released may be trapped and stored in the pyrophosphate linkages of adenosine triphosphate (ATP), the structure of which is shown in Figure 1. It has long been known that ATP is a compound of central importance in the cell and that it acts as a reservoir of energy for the performance of muscular work, synthetic reactions, and other vital activities; and a study of the enzymatic reactions which we will now discuss has made it

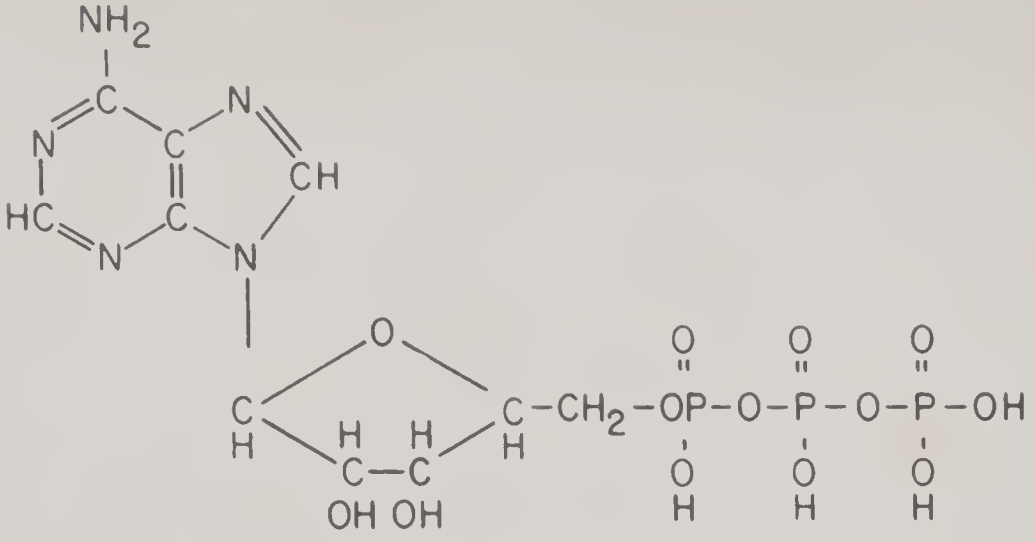

Figure 1. Structure of adenosine triphosphate (ATP).

clear that the oxidation of fats leads to the regeneration of this important substance in the body.

MECHANISM OF FATTY-ACID OXIDATION

A scheme depicting the β-oxidation of fatty acids as carried on by animal cells is shown in Figure 2. The essential participation of coenzyme A (CoA),

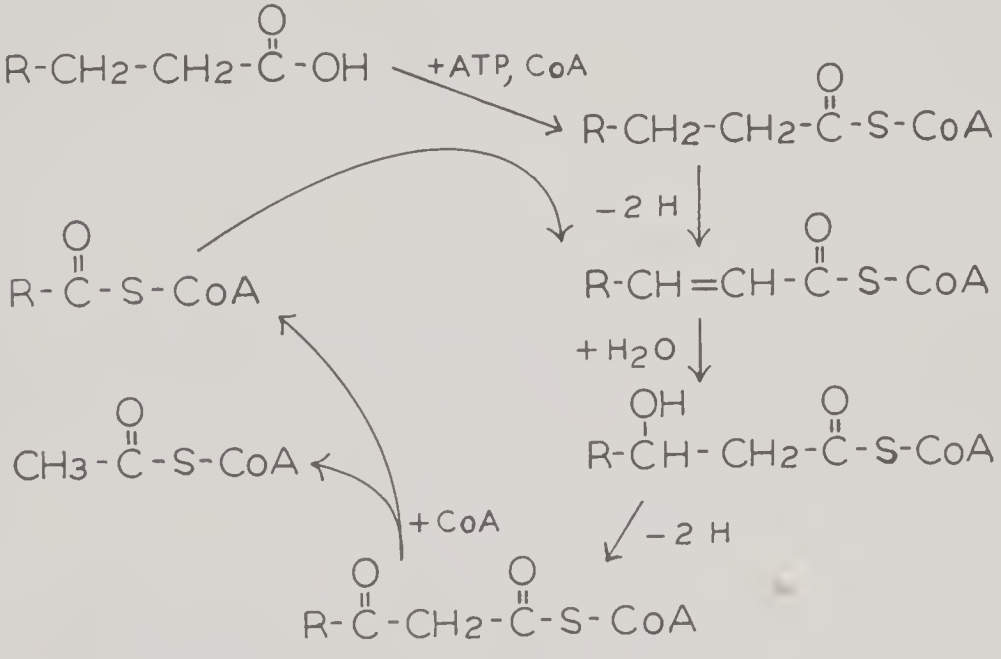

Figure 2. Reactions of the fatty-acid cycle.

the pantothenic acid-containing cofactor discovered by Lipman, is at once apparent. In fact, properly speaking, the fatty acids themselves do not undergo oxidation; it is only in the form of CoA thioesters that these reactions occur. As shown here, the initial step in the oxidation of the fatty-acid molecule is the conversion to a CoA thioester, a reaction which actually uses, rather than forms, ATP. It is thus necessary to "spark" or "prime" the system in order to initiate the oxidative attack.

The fatty-acid thioester of CoA is then dehydrogenated in a step catalyzed

by enzymes known as acyl dehydrogenases. At least three such enzymes are now known, each specific for a different range of chain length of fatty acid. The coenzyme of the acyl dehydrogenases has been identified as flavin adenine dinucleotide (FAD). The product formed at this stage is an α,β-unsaturated derivative, as shown.

In the next stage the double bond is hydrated by an enzyme called enoyl hydrase, forming an L-β-hydroxyacyl CoA. This compound is then enzymatically dehydrogenated, forming a β-ketoacyl CoA in a reaction catalyzed by a specific dehydrogenase, utilizing diphosphopyridine nucleotide (DPN) as electron acceptor. The β-ketoacyl derivative then reacts with another molecule of CoA, with the formation of acetyl CoA and a CoA ester of a fatty acid containing two carbon atoms less than the original fatty acid. This shorter CoA thioester then undergoes the same series of reactions until all the molecule has been split into acetyl CoA molecules. Thus the carbon chain is degraded two carbon atoms at a time.

Acetyl CoA, formed during the process of fatty-acid oxidation, may participate in a number of synthetic processes of vital importance to the cell, or the acetyl moiety of this compound may be completely oxidized to carbon dioxide and water by way of the rather complex cyclic mechanism known as the Krebs cycle.

GENERATION OF ATP

In the reactions shown in Figure 2, no mention is made of the generation of ATP. However, at two points coenzymes are reduced, namely, at the acyl dehydrogenase step and at the β-hydroxyacyl dehydrogenase step. Furthermore, during the oxidation of acetyl CoA via the Krebs cycle, coenzymes are reduced at four points, and at one step a molecule of ATP is generated directly during the conversion of α-keto-glutaric acid to succinic acid. Largely as a result of the work of Lehninger, it has been found that the reoxidation of reduced coenzymes in the cell is coupled to the regeneration of ATP. This process, called "oxidative phosphorylation," involves several intermediate steps, culminating with the eventual reduction of molecular oxygen to water. The mechanism of this process and the manner in which ATP is generated during it are not completely known at present and are the subject of intensive investigation in several laboratories.

The number of ATP molecules which can be regenerated by the oxidation of reduced coenzymes has been determined experimentally. The pyridine nucleotides, such as DPN, yield three, and the flavin coenzymes yield two, molecules of ATP. From these data one can calculate the yield of ATP per mole of palmitic acid oxidized according to the scheme in Figure 2 and the Krebs cycle. This turns out to be 131 moles of ATP per mole of palmitate, minus the 1 mole of ATP needed to initiate the process, or a net yield of 130 moles per mole of palmitate. In comparison, glucose derived from carbohydrate yields only 38 moles of ATP per mole when it is completely oxidized to carbon dioxide and water.

This high yield of ATP thus furnishes an explanation in well-defined chemical terms of the importance of fats as energy-rich foods. Although the exact amount of energy made available to the cell per mole of ATP is still the subject of debate, it can be calculated that about half the maximal free energy released in equation (1) may actually be captured as ATP, a figure which represents an impressively high efficiency. It should be made clear that these calculations are based on optimal yields of ATP at each step; it is not known if the cell always operates at optimal efficiency or whether it always converts all the energy available from fatty-acid oxidation into ATP.

OXIDATION OF ESSENTIAL FATTY ACIDS

Since the interest of this audience may be especially directed toward the metabolism of essential, polyunsaturated fatty acids, it may be appropriate to make some observations on the oxidation of this type of fatty acids.

Studies by Kennedy and Lehninger (4) several years ago revealed that polyunsaturated fatty acids such as linoleic and linolenic acids are readily oxidized in vitro by enzyme systems of the type under consideration here. This was somewhat surprising. It had been thought that the metabolism of these compounds might well present special aspects, since nutritional studies had indicated that experimental animals exhibit gross symptoms of essential fatty-acid deficiency only after rather prolonged periods on a diet low in these compounds. This observation might suggest that the body conserves polyunsaturated fatty acids by some special mechanism; however, experiments with the in vitro system seemed to indicate that there was little difference between the oxidation of saturated and polyunsaturated fatty acids in the rat. More recently, Mead and his collaborators (5) have shown that labeled linoleic acid when fed to rats is actually oxidized at a rate somewhat faster than stearic, at least under the experimental conditions employed. These results confirm the earlier work in vitro and suggest that the conservation of essential fatty acids must take some form other than a diminished rate of oxidation.

SYNTHESIS OF FATTY ACIDS

The synthesis of fatty acids from acetyl CoA takes place essentially by reversal of the reactions shown in Figure 2. There are several points of difference between oxidation and synthesis, however. Oxidation occurs almost entirely in the intracellular particles known as mitochondria. These particles contain the enzymes for the steps in Figure 2, for the Krebs cycle, and also for oxidative phosphorylation. They serve as a sort of "ATP factory," supplying ATP for the cell. The synthesis of fatty acids probably occurs in the cytoplasm of the cell, on the other hand (6). The reactions in Figure 2 run backward, with the minor exception that at the acyl dehydrogenase step a pyridine nucleotide (TPN) instead of a flavin serves as coenzyme. The synthesis of a mole of palmitic acid thus requires 8 moles of acetyl CoA and 14 moles of reduced pyridine nucleotides (DPN and TPN). The acetyl CoA and reduced coenzymes necessary for this process can be generated by the partial oxidation of carbohydrates by enzymes in the cytoplasm. Reduced DPN is formed by glycolysis of glucose to pyruvate and the oxidation of pyruvate to acetyl CoA. Reduced TPN may be formed by the oxidative reactions of the hexose monophosphate shunt. Nearly 5 moles of glucose must be oxidized in order to synthesize 1 mole of palmitate, since some carbon is lost as carbon dioxide, but the process is quite efficient. A balance sheet shows that the conversion of 5 moles of glucose to 1 mole of palmitate and 14 moles of carbon dioxide can cause the regeneration of 50 moles of ATP, together with the potential for 130 moles of ATP stored in the palmitate molecule. This total of 180 moles of ATP compares favorably with the 190 moles of ATP that could be obtained by the normal complete oxidation of 5 moles of glucose to carbon dioxide and water. Thus it can be seen that fatty-acid synthesis from carbohydrate provides an efficient method for storing the potential for forming ATP in a compact form that can be used at a later time.

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VII

GROWTH-PROMOTING PROPERTIES OF MEAT MEAL

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When weanling rats are fed a diet complete with respect to known vitamins, salts, etc., but containing 1 per cent sulfaguanidine, growth is inhibited almost completely by the fifth week. The rats exhibit typical symptoms of a thyroid deficiency. If a purified diet is used, death invariably results in 12-14 weeks. On a more natural dietary regime, growth is inhibited, but the animals do not die, at least through a 6-month period.

If meat meal, at a level of 5 per cent, is incorporated into the diet, growth and appearance of the rats are normal. A typical experiment to test the effectiveness of meat meal and skim milk is shown in Figure 1. When it became apparent in this experiment that meat meal, but not skim milk, stimulated growth, meat meal (10 per cent) was added to the diet of one of the growth-inhibited groups to test whether the condition could be reversed by meat meal. The other group was retained as a control. Meat meal definitely stimulated growth of these rats.

From this experiment a curative-type growth assay was devised to test the effectiveness of other substances to stimulate growth of sulfaguanidine-fed rats. This consists of feeding weanling rats a complete diet containing 1.1 per cent sulfaguanidine until growth is less than 5 gm. per week (4-5 weeks). Then the substance to be tested is added at the expense of the entire diet and fed for 2 weeks. A gain of 10 gm. or more during this period is an indication of growth-promoting activity of the sample. Three rats per group are usually used, one male and two females. This is because it had been occasionally observed that a rat will continue to grow on the "deficient" diet, and this occurred more frequently in males than in females. The female rat responds slower than the male, but growth of the female is a better criterion of response to the diet fed.

It has been reported (1) that meat-meal ash; egg; milk; liver from hog, calf, and steer; and feces from the rat, cow, or chicken failed to stimulate growth of sulfaguanidine-fed rats. Plant substances such as wheat, peanut meal, soybean-oil meal, and corn were also ineffective. Doubling the level of all known B vitamins failed to stimulate growth. Only meat meal or 0.02 per cent thyroid powder stimulated growth.

Several laboratories (2-6) have demonstrated the goitrogenic properties of sulfonamides, thiourea, and thiouracil and the antigoitrogenic properties of thyroxine. Vitamin deficiencies produced by sulfonamides have been overcome by liver or liver concentrates (5,6). However, Axelrod et al. (5) reported that growth inhibition of rats fed 0.5 per cent sulfaguanidine could be overcome by p-aminobenzoic acid, biotin, and liver fractions, but these had only a transient effect when 1.0 per cent sulfaguanidine was fed. Ershoff (6) suggested that liver contained an unknown growth factor which stimulated growth of thiouracil-fed rats. Only two out of seven samples of liver were effective in this respect.

Since the activity of meat meal was destroyed by alkaline hydrolysis and it was stable to short periods of acid hydrolysis, it was concluded that thyroxine was not the active principal in meat meal (1). Thyroxine is stable to acid and alkaline hydrolysis (7). Also an attempt was made to isolate thyroxine from

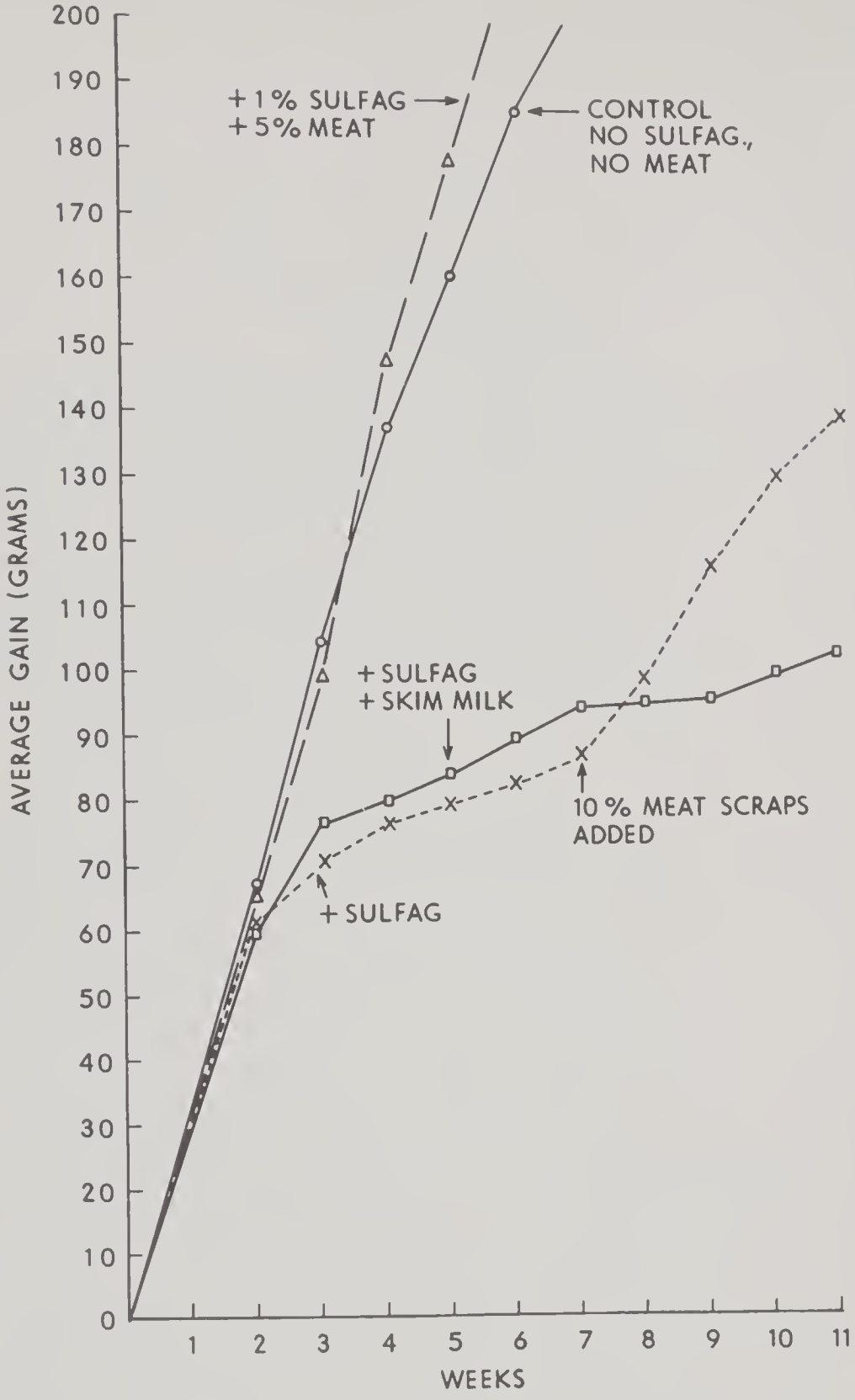

Figure 1

meat meal by the procedure of Kendall (7). Fractions from various steps of the procedure were tested for activity without success, but, when this procedure was applied to fresh beef thyroid, the small amount (about 20 mg.) of crude thyroxine caused thyrotoxicosis and death when assayed for activity.

It became of interest to determine whether meat meal could overcome the growth inhibition of thiourea and thiouracil, since these substances are not considered as bacteriostatic agents and not likely to interfere with the growth

of the intestinal flora. Ten groups of four weanling rats each were fed 1 per cent sulfaguanidine, 0.1 per cent thiourea, or 0.1 per cent thiouracil with and without 5 per cent meat meal. Two groups were fed 0.015 per cent thyroid powder, with and without sulfaguanidine, to determine the effect of thyroid powder on the weight of the thyroid gland. The results after 6 weeks of feeding are shown in Table 1. Growth of rats receiving 0.1 per cent thiourea and thiouracil was definitely retarded, but the addition of meat meal stimulated growth, although not to optimum. Meat meal also tended to decrease the size of the thyroid gland, but again not to normal levels. The addition of thyroid powder resulted in slightly better than normal growth and normal thyroid weights. Presumably, meat meal did not completely overcome the toxicity of thiourea and thiouracil, but normal growth was achieved when meat meal was fed with sulfaguanidine.

TABLE 1

Effect of Meat Meal on Goitrogen-fed Rats

(Four Rats per Group Fed ad libitum from Weaning)

Diet Addition	6-Week Gain (Gm.)	Thyroid Wt. (Mg./100 Gm. B.W.)
None	164	7.1
5% meat	175	7.2
1% sulfaguanidine	84	33.7
1% sulfaguanidine + 5% meal	173	14.0
0.1% thiourea	53	19.8
0.1% thiourea + 5% meat	93	14.8
0.1% thiouracil	102	26.9
0.1% thiouracil + 5% meat	134	21.2
0.015% thyroid pwd.	189	6.2
0.015% thyroid pwd. + 1% sulfaguanidine	194	7.3

This suggested an examination of protein-bound iodine (PBI) of blood and iodine levels of the thyroid gland. Three groups of six weanling rats were fed the following diets: (I) control; (II) 1 per cent sulfaguanidine; and (III) 1 per cent sulfaguanidine plus meat meal. After 6 weeks the animals were sacrificed and the thyroid glands removed and weighed. Protein-bound iodine of the plasma and total iodine of the thyroid glands were determined. Results are shown in Table 2, Group A. When meat meal plus sulfaguanidine were fed, growth was normal, but PBI of the plasma and total iodine of the thyroid gland was less than that of the growth-inhibited rats which were fed 1 per cent sulfaguanidine. This experiment was completed in April, 1957, but the experiment was repeated in September, 1957, except that eight rats per group were used. Four rats from each group were used for iodine determination, and histological examination of the thyroid glands was made on the remaining four rats by J. R. Rooney II, professor of animal pathology, Department of Animal Pathology, Virginia Polytechnic Institute. In this experiment, rats fed meat meal plus sulfaguanidine grew somewhat less than the normal controls (Table 2, Group B), but, again, PBI of plasma and total iodine of the thyroid gland were less than that of the growth-inhibited rats. Also marked hyperplasia of the thyroid glands was observed histologically in both groups of sulfaguanidine-fed rats. It would seem that the animals fed sulfaguanidine plus meat meal are growing in spite of the lack of thyroid hormone and that growth is stimulated by some factor in

TABLE 2

Effect of Meat Meal on Plasma PBI and Total Iodine of Thyroid Gland

Diet	Av. Gain (Gm.)	Plasma PBI μGm. 1/100 Ml.	Total I of Thyroid / μ Gm.	μGm. 1/100 Ml. / Mg. Thyroid Wt.
	Group A (April)*			
Normal	168	6.10	1,151	79.4
1% sulfaguanidine	64	3.67	882	29.8
1% sulfaguanidine + 5% meat	170	2.59	488	15.5
	Group B (September)†			
Normal	181	5.13	675	56.8
1% sulfaguanidine	69	5.88	493	17.9
1% sulfaguanidine + 5% meat	153	3.67	247	8.3

*Data from six rats per group maintained for 7 weeks.

†Average weight gain of eight rats per group, but iodine analysis is an average of four rats per group.

meat meal which is not thyroxine.

It was desirable to determine which tissues were responsible for the growth activity of meat meal. Various dried samples of tissues were obtained from Hudson Pharmacal Company, Armour and Company, Viobin Corporation, and Wilson & Co., Inc. These were tested by the 2-week curative-type growth assay. Table 3 is a summary of the growth-promoting property of these tissues, and in some cases the thyroid weights are given, calculated as milligrams of thyroid weight per 100 gm. of body weight.

In general, it appears that tissues associated with digestion are active. This includes the stomach, pancreas, duodenum, and an extract of hog intestinal mucosa. Tissue B (a preparation of brain and spinal cord) is the exception, and, since brain was inactive, this requires further investigation. The pyloric section (two samples) of the stomach was inactive, and the question arises as to whether the activity of the stomach is due to the secretory section or to fundus. Mammary tissue was slightly active, but fish meal and fish flour were inconsistent in their activity. Experience suggests that preparation and treatment of samples may be an important factor that determines the activity of the tissue. Two different preparations of hog stomach and three fish samples were inactive, and separate experiments with fresh hog stomach and pancreas were inactive after simply drying the tissues at 90°F. in a forced-draft oven. The activity of meat meal is little affected by such treatment (1). It should also be mentioned that meat meal purchased in Alabama and Virginia over the last five years has been consistently active. It is estimated that from ten to twenty 100-pound lots of meat meal have been used during this period. Not one has been inactive.

SUMMARY

Meat meal, hog pancreas, hog stomach, and certain other tissues prevent

TABLE 3

Growth-promoting Effects of Some Tissues on Sulfaguanidine-fed Rats*

Diet Additions	2-Week Gain (Gm.)	Thyroid Wt. (Mg./100 Gm. B.W.)
Hog stomach (Hudson)	45	-
Hog stomach (Hudson)	7	17.0
Hog stomach (Armour)	32	-
Hog stomach (Armour)	45	7.8
Hog stomach (Wilson)	9	40.7
Hog pancreas (Armour)	41	-
Hog pancreas (Wilson)	60	13.5
Hog duodenum (Hudson)	32	17.6
Hog duodenum (Wilson)	29	35.1
Hog intest. ext. (Armour)	39	-
Hog pyloric (Hudson)	5	-
Ext. hog pyloric (Armour)	-5	-
Liver residue (Wilson)	9	40.1
Whole liver (Wilson)	2	40.8
Kidney (Wilson)	5	48.9
Brain (Wilson)	4	44.9
Mammary (Wilson)	17	43.9
Spleen (Wilson)	48	22.3
Tissue B (Wilson)	49	17.1
Fish meal (Viobin)	4	-
Fish meal (Viobin)	31	24.3
Fish meal (Viobin)	2	21.9
Fish flour (Viobin)	5	33.3
Fish flour (Viobin)	37	22.7
Fish meal (Redville)	2	36.2
None	6	34.2

*Rats fed 1.1 per cent sulfaguanidine from weaning until growth ceased; all substances added as dry powder—at a level of 5 per cent.

or cure the growth inhibition of rats fed sulfonamides. The active principal does not appear to be thyroxine, since it is alkali labile and does not prevent hyperplasia of the thyroid gland. Also the protein-bound iodine level of the blood serum and total iodine levels of the thyroid gland are less than that of growth-inhibited rats fed sulfaguanidine. It is suggested that meat meal contains a growth-promoting principle which is normally produced by some tissue, possibly the pancreas. The synthesis of this factor is controlled by the thyroid hormone, and the administration of goitrogenic agents inhibits the synthesis of the thyroid hormone and, thus, the growth-promoting factor.

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VIII

INFLUENCE OF PRESLAUGHTER FEEDING AND HOLDING ON MEAT QUALITY[1]

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Research by Meyer and co-workers (10) has indicated that there is extracellular and intracellular fluid loss in animal tissue during time of stress. Bjorka (4) reported that excitement and fatigue, owing to stress, are important factors in the weight loss of livestock during shipment and in the stockyards. Klussendorf (9) reported that the net result of stress is that body requirements are above and beyond the usual or average needs.

Ashby (2) found considerable variation in the shrinkage of "truck" hogs. This shrinkage tended to increase with length of haul. Hogs not fed at market showed heavier shrinkage than did hogs that were fed. Hedrick et al. (8) reported that all animals do not react alike to a given stress. Muscle glycogen of a normal animal is not depleted in a matter of minutes or even an hour or two. Robison (11) reported hogs fasted the morning of shipment were found to shrink less in transit than those given a normal feed the morning they were shipped.

Research by Heck (7) indicates that an opportunity exists to reduce weight loss and improve the quality of pork by preslaughter feeding and holding.

Callow and Ingram (5) report the various factors which may give rise to bone taint. They suggest that the outbreaks, which are often sporadic and puzzling in nature, may be due to bacteria passing through the wall of the gut, into the blood stream, and so into the muscles, even in living animals. They also report that there is much evidence, mostly from medical experience with humans, that certain conditions besides disease are likely to cause this. These factors are fatigue, fright, shock, or even a sudden strain. They report that the most important aspect of the invaded tissue is their pH, because most species of bacteria concerned prefer a neutral or slightly alkaline environment, and that their growth is retarded by even slight acidity. It is suggested by Callow and Ingram (5) that fatigue of the live pig reduces the glycogen reserve of some of the muscles and that the higher ultimate pH of these muscles after death offers less resistance to bone-taint organisms.

Bate-Smith and Bendall (3) report that the initial pH of the muscle is shown to be mainly dependent on the severity of the death struggle, whereas the ultimate pH is determined by the level of feeding and degree of fatigue of the animal immediately before death.

Bacterial growth was found by Gibbons and Rose (6) to be considerably reduced if the pH of the meat was below 5.6. They report that the correlation (.70) between pH and lactic acid content of the muscle suggests that lactic acid is the most important single factor influencing the pH. Callow and Ingram (5) report that lactic acid is the natural preservative of meat. Bate-Smith and Bendall (3) report that the chief source of lactic acid is carbohydrate and that the

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1. Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

problem of obtaining meat of low pH becomes primarily one of maintaining a high reserve carbohydrate (glycogen) level in the muscles.

Proper preslaughter feeding and holding may allow the carbohydrate (glycogen) level to increase and thus maintain a higher level in the muscles during slaughtering.

EXPERIMENTAL

Twelve barrows averaging 229 pounds were randomized into two lots and fed a preslaughter feed consisting of shelled corn and water. Lot I received a high concentrate carbohydrate (brown sugar) in the drinking water at the rate of 3 pounds per 5 gallons. The water was hand-fed in steel troughs and was not withheld before slaughter. The grain was withheld 6 hours prior to slaughtering. The hogs were weighed 48 hours prior to slaughtering and again just before loading. They were trucked about 1 mile to the Meat Laboratory, where they were slaughtered.

Urine samples were collected from the six hogs fed sugar, and these samples were tested with Benedict's solution for glucose.

The pH of the stick blood, carcass weights, and liver weights were recorded.

Hams and shoulders were dry-sugar-cured for 3 weeks at 38°F. Then a random half was removed to an unrefrigerated room where the temperature averaged 46°F. The hams and shoulders cured at the high temperature for 7 days. They were wrapped for insulation and protection. The cuts were returned to the 38°F. room and allowed to cure another week, making a total of 5 weeks in cure. Weight losses during curing and smoking were recorded.

Center slices were removed and cooked for organoleptic panel testing. The samples were scored for the following quality factors: aroma, flavor of fat and lean, texture, tenderness, and juiciness. The higher the numerical score, the more acceptable was the sample.

RESULTS AND DISCUSSION

The preslaughter feeding of shelled corn and a high-concentrate carbohydrate in the drinking water 48 hours before shipping significantly lowered the live-weight loss (Table 1).

Urine samples from the sugar-fed hogs did not react positively with Benedict's solution. This indicated that the glucose had been absorbed.

There was little difference in the pH of the blood at time of bleeding. The hogs receiving sugar had an average pH of 7.4 compared to an average pH of

TABLE 1

Effect of Preslaughter Feeding on Live Shrink

Lots	No.	Initial Live Wt.	Final Shrink Wt.	Per Cent Loss
Treated	6	227.2	226.7	.22
Control	6	230.8	220.5	4.46
Difference	-	3.6	6.2	4.24**

**Significant at the 1 per cent level.

7.3 for the controls. The hot-carcass weights were the same; however, the sugar-fed hogs shrank more in chilling. This greater chill shrink, 2.6 per cent as compared to 1.8 per cent, apparently was responsible for a significant difference ($P < 0.01$) in the yield (Table 2). The bladders and small intestines of the sugar-fed hogs contained more fluids than normal.

TABLE 2

Effect of Preslaughter Feeding on Yield

Lots	No.	Hot-Carcass Wt.	Chill-Carcass Wt.	Per Cent Shrink	Per Cent Yield
Treated	6	173.8	169.3	2.6	74.7
Control	6	173.8	170.6	1.8	77.4
Difference	-	0.0	1.3	0.8*	2.7**

*Significant at the 5 per cent level.

**Significant at the 1 per cent level.

Liver weights averaged 0.4 of a pound heavier on the sugar-fed hogs, although the difference was not significant.

Weight records of hams and shoulders during curing and smoking revealed no significant differences. There was no spoilage during curing or smoking of hams or shoulders.

There was no significant difference among ham samples tested by an organoleptic panel for aroma, flavor of fat and lean, texture, tenderness, and juiciness. It was noted, however, that the judges scored all quality factors higher on hams from the sugar-fed hogs with the exception of flavor of lean (Table 3).

TABLE 3

Organoleptic Scores for Quality Factors of Hams

Lots	No.	Aroma	Flavor		Texture	Tenderness	Juiciness
			Fat	Lean			
Treated	6	80.5	80.6	81.5	86.6	83.5	77.8
Control	6	80.3	76.7	83.2	77.8	75.7	72.8
Difference	-	0.2	3.9	1.7	8.8	7.8	5.0

They scored shoulder samples from the hogs preslaughter-fed the high-concentrate carbohydrate significantly higher for flavor of lean ($P < 0.05$), texture ($P < 0.01$), and tenderness ($P < 0.01$) (Table 4).

TABLE 4

Organoleptic Scores for Quality Factors of Shoulders

Lots	No.	Aroma	Flavor		Texture	Tenderness	Juiciness
			Fat	Lean			
Treated	6	77.2	77.6	79.8	77.0	78.6	82.4
Control	6	70.8	72.8	71.2	66.4	66.3	78.9
Difference	-	6.4	4.8	7.6*	10.6**	12.3**	3.5

*Significant at the 5 per cent level.

**Significant at the 1 per cent level.

SUMMARY

The effect of a high-concentrate carbohydrate (brown sugar) in the drinking water on live and carcass shrink and factors of eating quality were studied.

Difference in live shrinkage was 4.24 per cent in favor of the preslaughter-fed hogs. It was observed that warm-carcass weights were the same and that yield favored the untreated hogs by 2.7 per cent.

Organoleptic scores for eating quality were inconsistent but tended to favor the cuts from the hogs receiving the high-concentrate carbohydrate feed.

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IX

TRANQUILIZERS IN ANIMAL FEEDS

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INTRODUCTION

Tranquilizers comprise a group of drugs which allay anxiety or tension without deadening pain or inducing sleep. They are being increasingly used in human medicine. A recent list of tranquilizers for humans[1] presents about a dozen and a half trade-named products which fall into four major chemical categories: (1) rauwolfia alkaloids; (2) derivatives of phenothiazine; (3) diphenylmethane derivatives; and (4) substituted propanediols. Many more are under investigation.

For use with animals the number of commercially available tranquilizing drugs is somewhat less. In Table 1 are given some on which information is available. They do not all have parallel pharmacologic properties, and it would exceed the scope of this paper to categorize them. Let it suffice to mention the following important uses in the hands of the veterinarian: to quiet and render tractable nervous, unfriendly, or unco-operative patients; to prepare for anesthesia with general anesthetics and even to use alone as anesthetics; to ease shock originating from a variety of causes; to stop convulsions; and to control vomiting.

In connection with the handling of animals by livestock-raisers, tranquilizers offer several benefits. They have been used to help distraught sows to accept their pigs; to calm calves at weaning as well as freshening heifers unused to machine milking; to diminish bawling and fence running by feeder calves in strange lots; and to pacify bulls, stallions, and other sometimes dangerous animals.

Another use is to quiet livestock before they are to be loaded for shipping other than to slaughter. Tranquilization of cattle in transit could reduce bruising and may help minimize shipping fever by diminishing nervous fatigue. Shrinkage in transit is said to be less in tranquilized animals. There has been interest also in whether tranquilizers given a short time prior to slaughter can reduce the number of "dark cutters"—cattle whose meat looks abnormally dark as a result of excitement attendant upon killing. If tranquilizers are effective but only in doses so large as to leave residues considered to be unsafe, this procedure may not be practical.

For poultry, results of studies reported to date are difficult to summarize briefly. A wide range of levels has been used in different trials and with different species, and there are some inconsistencies.

Garren and Hill (Poultry Sci., 36:1386-87 [1957]) fed meprobamate to Leghorns at levels of 250 and 500 gm. per ton. It did not lessen the nervous character of the birds and retarded weight gains. They found the same for promazine. Babcock and Taylor (Poultry Sci., 36:485 [1957]) fed from 0.2 to 1.8 per

1. C & E News, August 18, 1957.

TABLE 1

Tranquilizers for Veterinary Use

Doses Recommended by Manufacturers
(Mg. per Pound Body Weight)

Compound	Canine	Feline	Equine	Bovine	Ovine	Porcine
	Intravenous					
Thorazine	0.25-2.0	0.25-2.0	0.1	0.1-0.5	0.25-2.0	-
Trilafon	0.25-1.0	0.25	-	0.075*	-	0.1†
Diquel	1.0-2.0	1.0-2.0	-	0.25-0.50	-	-
Paxital	2.5-3.3	-	-	-	-	-
Sparine	1-3	1-3	0.2-0.5	0.2-0.5	0.2-0.5	0.2-0.5
Serpasil	0.02-0.04	0.00004-0.0002	0.002-0.005	0.005-0.01	0.01-0.02	-
	Intramuscular					
Thorazine	0.5-3.0	1.0-3.0	0.5-1.8	0.5-1.0	1.0-3.0	-
Trilafon	0.25-1.0	0.25	-	0.09*	-	0.1
Diquel	2.0-5.0	2.0-5.0	-	0.5-1.0	-	-
Paxital	2.5-3.3	-	-	-	-	-
Sparine	1.0-3.0	1.0-3.0	0.2-0.5	0.2-0.5	0.2-0.5	0.2-0.5
Serpasil	0.02-0.04	0.00004-0.0002	0.002-0.005	0.005-0.01	0.01-0.02	-
	Oral					
Thorazine	1.5	1.5	-	-	1.0	-
Trilafon	0.4-1.6	0.8	-	-	-	-
Diquel	2.0-5.0	2.0-5.0	-	-	-	-
Paxital	7.5-13.2	-	-	-	-	-
Sparine	1.3-3.9	1.3-3.9	0.205-0.66	0.205-0.66	0.205-0.66	0.205-0.66
Equanil‡	20.0-60.0	20.0-60.0	20.0-60.0	20.0-60.0	20.0-60.0	20.0-60.0
Serpasil	0.009-0.02§	0.00004-0.0002	0.004	-	0.2-0.3§	-

*<800 per lb. body wgt., 0.1 mg. per lb.

†Preferred route.

‡Split dosage.

§For 2-3 days.

cent of the ration. There was a slight, and probably not significant, weight increase at 0.2 per cent (1800 gm. per ton), but all other levels depressed growth. Crawford (Southern Ag. Workers Conf. [1958]) tested levels up to 250 gm. per ton. There was a growth stimulus at 25 gm. and higher.

Crawford (ibid.) reported that reserpine at both 25 gm. and 250 gm. per ton stimulated growth of chicks. Carlson (Proc. South Dakota Acad. Sci., 35:186 [1956]) fed turkeys levels equivalent to 0.46 and 0.92 gm. per ton. This diminished fighting in mixed groups of toms and hens but also decreased gains. Hewitt and Reynolds (Proc. Cornell Nutr. Conf. [1957], p. 53) fed 5 gm. per ton of feed to pheasants. This reduced feather picking but also reduced rate of gain somewhat. Van Matre, Burger, and Lorenz (Poultry Sci., 36:1165 [Abstr.]) found that reserpine (and also chlorpromazine) prolonged survival of chicks under high temperature, although not those less than 4 weeks. For those over 4 weeks, effective doses were equivalent to 90-450 gm. per ton of feed; for those beyond 7 weeks doses in the range of 23-46 gm. per ton sufficed. Both drugs protected against decrease of egg production and shell quality under heat stress. There was no mention of effects on growth.

TRANQUILIZERS AS GROWTH PROMOTANTS FOR RUMINANTS

A number of physiological mechanisms enter into growth of animals. Aside from genetic influences, the earliest investigations, of course, were directed

toward determining quantitative relationships between weight gains and intake of macronutrients (protein, carbohydrate, fat, certain minerals) which actually become part of the tissues of the organism or exert indirect influences upon metabolism. Then came under scrutiny the micronutrients—vitamins, unidentified growth factors and, more lately, trace minerals. Quantitative requirements were defined for these for several species, including animals of economic importance. Refinements in quantitative relationships still are being worked out. Simultaneously, physiological functions of a number of micronutrients have been elucidated in some detail.

An obvious area to investigate when studying growth was the influence of hormones. This early attracted the attention of biologists and physiologists. A vast array of information has been developed, much of which is useful in human medicine and veterinary medicine. In commercial operations, perhaps the most outstanding application of hormones has been the use of estrogens to improve productive performance of poultry and ruminants. For these species, the effects of estrogens, natural or synthetic, given orally or by implantation, have been evaluated quantitatively in numerous trials. Effects of estrogens upon growth of other mammalian farm animal types are less well defined, and thus far commercial applications have not been made, but much research is under way.

Although discovered much later than the hormones, the antibiotics attained more widespread attention as growth promotants. In general, the antibiotics have been found effective agents for speeding growth of all major types of meat animals and meat birds, and, it may be remarked, they are applied also in feed supplements to sustain and increase egg production throughout the laying cycles of chickens and turkeys. The mechanism of growth promotion by antibiotics is still not fully understood, although preponderance of evidence suggests that it is by influencing intestinal microflora.

Hormones and antibiotics often are used simultaneously in the feeding of beef cattle and sheep. In numerous trials the growth increment provided by simultaneous feeding of both additives has been found to exceed the increment observed when each has been fed separately. This indicates separate mechanisms of growth promotion. It should be added that there may be synergism as, in some trials, the growth increment from the combined use has exceeded the sum of the increments from each separately.

The discovery of a growth-promoting effect of certain tranquilizers was made accidentally when graded oral levels of such drugs were being tested to quantify their sedating action. The growth stimulus in ruminants is provided by levels far below those which bring about sedation. Moreover, a growth increment has been brought about by tranquilizers in the presence of optimum supplementation with antibiotics and/or hormones, indicating still another growth mechanism made to operate more intensely by this, the latest type of feed additive in commercial use or nearing that stage. Mainly because of their newness, less is known of the mode of action of tranquilizers than of antibiotics or hormones.

QUANTITATIVE GROWTH STUDIES WITH TRANQUILIZERS

Lambs

At the Pfizer Agricultural Research Center the major investigations of tranquilizers to date have been made with cattle and sheep.

Table 2 compares graded levels of Tran-Q[2] in growth of wether lambs all of which received an implant of stilbestrol and oral Terramycin.[2]

2. Trade mark, Chas. Pfizer & Co., Inc.

TABLE 2

Comparison of Graded Levels of Tran-Q in Growth of Wether Lambs

(Pfizer Trial 16.1-14 [69 Days])

All Groups Received 3 mg. Stilbestrol Implant and 50 mg. Terramycin per Head per Day

	No. Lambs	Gain (Lb.)	Incr. (Per Cent)	Lb. Feed/ Lb. Gain	Impr. (Per Cent)	Dress. (Per Cent)	Grade
Control	24*	0.477	-	7.35	-	48.83	7.91
Tran-Q, 0.24 gm./ton	12	0.473	(-) 1	7.54	(-)2.5	47.53	7.50
Tran-Q, 1.2 gm./ton	12	0.547 †	15	6.98	5	49.12	8.18
Tran-Q, 6.0 gm./ton	12	0.498	4	7.19	2	48.40	8.17
Tran-Q, 30.0 gm./ton	12	0.485	2	7.31	0.5	47.70	8.33

RATION (Pelleted; Self-fed)

Ingredient	Per Cent
Sun-cured alfalfa	64.9
Yellow corn meal	25.0
Molasses	10.0
A-5 P (vitamin A)	0.1
Total	100.0

*Two replicates.

† $P > 0.05$.

Two unpublished Pfizer lamb trials can be summarized as follows:

Trial 16.1-15 (70 days): This was a comparison of stilbestrol, 3 mg. implant; Terramycin, 15 gm. per ton feed; and Tran-Q, 2 mg. per ton. Increments in average daily gains were 39, 22, and 28 per cent for these three treatments, respectively. Corresponding feed efficiency improvements were 32, 14, and 22 per cent.

Trial 16.1-21, 1.1-238 (83 days): This was a comparison of stilbestrol, 3 mg. implant; Terramycin, 15 gm. per ton, and Tran-Q at 1.2 gm. and 2.0 gm. per ton. Increments in average daily gains were 28, 26, 42, and 17 per cent for the respective treatments. Corresponding improvements in feed efficiency were 21, 10, 8.9, and 2.4 per cent.

A study of several products as growth promotants for lambs, including Tran-Q, has been reported by Iowa State College (I.S.C.A.H. Leaflet 226 [Dec., 1957]). The study was made in the facilities of a large packing company, and the trial groups at the start numbered 200 lambs each. A condensation of the results with Tran-Q is shown in Table 3.

Another university study is summarized in Table 4.

The data on Tran-Q from available lamb trials are summarized in Table 5.

Levels above 2 gm. per ton seem to offer no advantage over lower levels, and, in fact, higher levels may be above optimum. Therefore, averages are presented only for the range from 1.2 to 2.00 gm. per ton. There is considerable variability, the growth results being much higher than any others in one of the 1.20 gm. tests. Nevertheless, there is a substantial positive growth increment and feed efficiency improvement in all six comparisons at 1.2 gm. to 2.0 gm. The extremely high growth increment in one trial should be held in mind when considering the averages of increments over controls for this range which are: growth, 21.2 per cent; feed efficiency, 8.0 per cent; carcass grade, +0.20; added financial return, $1.36 per animal. It is noteworthy that in Pfizer Trial 16.1-14, which includes one comparison at 1.2 gm., the controls and treated already had been stimulated to superior performance by Terramycin

TABLE 3

University Study of Tran-Q in Lamb Growth

Iowa State College Trial (50 Days)

	Control	Tran-Q*
No. lambs marketed	197	197
Av. daily gain, lb.	0.41	0.48
Increase, %	-	17
Lb. feed/lb. gain	8.23	7.71
Improvement, %	-	6
Cost/cwt. gain, $ †	17.00	16.66
Margin/lamb, $	0.83	1.09
Yield, %	47.17	46.98
Carcass grades:		
Prime	12	10
Choice	169	158
Good	15	27
Utility	1	1
Yearling Choice	-	-
Condemned	-	1

RATION (Not Pelleted)

Ground shelled corn	55.0
Corn molasses	10.0
Ground alfalfa hay	27.9
Linseed meal	7.0
Antibiotic supplement	1.0

*Tran-Q was added in a carrier included at 1.5 lb. per ton of feed. This was equivalent to 1.5 gm. per ton of the active ingredient (hydroxyzine).

†Comprises feed costs plus an arbitrary $4.00 for each lot to cover other costs.

ınd stilbestrol. Nevertheless, the tranquilizer induced still more intensive performance.

Beef Cattle Studies

In Table 6 appear results of feeding graded levels of Tran-Q to steers. The responses were not too closely related to dosage level, although there s an indication that the highest level exceeded the optimum. The negative financial return in that group was due to the considerable cost contributed by he high level of the drug.

Three unpublished Pfizer cattle trials have yielded the following results:

Trial 16.1-19 (169 days): Comparisons were between stilbestrol implant, 36 mg.; Terramycin, 75 mg. per head per day; and Tran-Q at 1.25 and 10 mg. per head per day. Increments over controls in average daily gains were 19.1, 5.6, 6.1, and 4.6 per cent for the respective treatments. Corresponding improvements in feed efficiency were 12.2, 0.0, 5.2, and 1.0 per cent. The response to Terramycin in this trial was somewhat less than average.

Trial 16.1-11;15.3-35 (84 days): This was a study of superimposing Tran-Q, 2.4 mg. per head per day, upon the following treatments: Terramycin, 80

91.92

TABLE 4*

University Study of Tran-Q in Fattening Lamb Rations (65 Days)

	Control	Tran-Q†
No. lambs‡	10	10
Av. daily gain, lb.	0.474	0.514
Increase, %	-	8
Feed per lb. gain	8.89	8.57
Improvement, %	-	4
Dressing, %	48.98	48.37
Carcass grade	9.7	10.0

RATION (Pelleted)

Ground alfalfa hay	30
Ground grass hay (poor quality)	30
Ground barley	28
Linseed oil meal	7
Beet molasses	5

*Church & Fox, Proc. 16th Ann. Oregon Animal Industry Conference, Feb. 6-7, 1958, p. 55.

†Tran-Q added at rate of 2 gm. per ton pelleted ration.

‡Replicate lots of 5 lambs each.

mg. per head per day; stilbestrol, 36 mg. implant; and Terramycin plus stilbestrol. The extra growth in the presence of the tranquilizer was as follows: No other treatment, 2 per cent; Terramycin, 9 per cent; stilbestrol, 17 per cent; Terramycin plus stilbestrol, 15 per cent. Corresponding feed efficiency improvements were 2, 7.5, 18.4, and 10 per cent.

Trial 15.3-29 (111 days): This was a comparison of Tran-Q, 2.4 mg. per head per day, with a control. Growth rate was increased by 9 per cent and feed efficiency by 5 per cent.

A state agricultural experiment station trial with Tran-Q is summarized in Table 7.

Another state agricultural experiment station trial appears in Table 8.

No signs of sedation were noted in the groups fed tranquilizers.

An extensive study with three tranquilizers was reported at the 1958 Indiana Cattle Feeders Day by Beeson, Perry, Andrews, and Stob of Purdue. There were two parts in the study. In one, the tranquilizers were used in the absence of other growth stimulants, and, in the other, all steers, including controls, also received a 36-mg. implant of stilbestrol and were fed 80 mg. of Terramycin per head daily.

As shown in Table 9, there was a distinct reponse to the tranquilizers when fed to animals which also received Terramycin and stilbestrol, the growth increment from 2.50 mg. Tran-Q per head being significant at the 5 per cent level. Feed cost per pound of gain was lowest in this group. The lack of response to tranquilizers, and especially to Tran-Q fed in the absence of other growth stimulants, is in contrast with positive findings under these conditions obtained in the several trials summarized on the preceding pages.

TABLE 5

Summary of Lamb Feeding Trials with Tran-Q

Source	No. Animals		Tran-Q (Gm./Ton)	Growth Incr. (%)	F. E. Impr. (%)	Carcass Grade Diff. from Contr.	Return over Contr. ($) Animal
	Contr.	Tran-Q					
Pfizer 16.1-14	24	12	0.24	(-) 1.0	(-) 2.5	(-) 0.41	(-) 0.42
Pfizer 16.1-21	24	12	1.20	42.0	8.9	+1.17	2.86
Pfizer 16.1-14	24	12	1.20	15.0	5.0	(-) 0.27	0.18
Iowa	197	197	1.50	17.0	6.0	(-) 0.10*	0.26
Oregon	10	10	2.00	8.0	4.0	+0.30	-
Pfizer 16.1-15	12	12	2.00	28.0	22.0	(-) 0.08	3.03
Pfizer 16.1-21	24	12	2.00	17.0	2.4	+0.28	0.50
Pfizer 16.1-14	24	12	6.00	4.0	2.0	+0.26	(-) 0.02
Pfizer 16.1-14	24	12	30.00	2.0	0.5	+0.42	(-) 0.93
Averages for 1.2 to 2 mg. range				21.2	8.0	+0.20	+1.36

*Basis of prime, choice, good.

TABLE 6

Effect of Graded Levels of Tran-Q on Growth of Steers

Pfizer Trials 16.1-16; 15.3-48 (112 Days)

All Animals Implanted with 36 mg. Diethylstilbestrol

	Tran-Q per Head Daily, mg.					
	0	1.25	2.5	10	50	500
No. steers	5	4	4	4	4	4
Av. daily gain, lb.*	2.25	2.83	2.68	2.82	2.74	2.61
Increase, %	-	26	19	25	22	16
Lb. feed/lb. gain	10.41	9.12	9.16	9.16	8.68	9.16
Improvement, %	-	12.1	12.0	12.0	16.7	12.0
Carcass grade	10.4	10.2	11.2	10.2	10.5	10.3
Dressing, %	59.77	61.54	61.22	60.47	60.57	60.16
Return over control, $	-	12.51	17.01	7.31	10.86	(-)8.47

RATION

Ground ear corn	Self-fed
Alfalfa hay	3 lb.
Supplement	2 lb.

*Average of all Tran-Q levels significant, $\underline{P} > 0.10$.

TABLE 7*

Trial of Tran-Q at Two Levels as a Growth Stimulant for Steers

Mississippi Agr. Exp. Sta. (111 Days)

	Control	Tran-Q	
		5 Mg./ Hd./Day	10 Mg./ Hd./Day
No. steers per lot	6	6	6
Av. daily gain, lb.	1.85	1.85	2.12
Increase, %	-	0	14
Av. carcass weight, lb.	594	583	609
Av. dressing percentage	59	60	61
Av. carcass grade†	9.83	9.16	10.17
Lb. feed/lb. gain‡	17.9	14.5	13.8
Improvement, %	-	19	23

*Personal communication, Dr. Bryan Baker.

†Grade code: high choice 14; av. choice 13; low choice 12; high good 11; av. good 10; low good 9; high standard 8.

‡The feeds used were crushed corn, cottonseed meal, and Johnson grass hay.

TABLE 8

Trial of Tran-Q and Chlorpromazine as Growth Stimulants for Steers

Oklahoma State University* (157 Days)

All Animals Implanted with Stilbestrol

	Control	Tran-Q†	Chlorpromazine‡
No. steers	10	10	10
Av. daily gain, lb.	2.22	2.32	2.25
Increase, %	-	4	1
Lb. feed/lb. gain	8.09	7.70	7.92
Improvement, %	-	5	2
Feed cost per cwt., $	17.60	16.73	17.15
Net return/steer, $	32.21	37.21	34.77

*L. S. Pope, R. F. Hendrickson, L. Walters, G. Waller, and W. D. Campbell. 32nd Annual Livestock Feeders Day, April 19, 1958.

†One mg./head daily for 90 days, then increased to 5 mg.

‡10 mg./head daily.

TABLE 9

Effect of Tranquilizers Fed with and without Stilbestrol and Terramycin for Fattening Steers

Purdue—Annual Livestock Feeders Day
April 27, 1958—175 Days

Lot No. and Treatment	Initial Wgt. (Lb.)	Daily Gain (Lb.)	Incr. (%)	Feed/Lb. Gain (Lb.)	Impr. (%)	Feed Cost/ Lb. Gain (Cents)
	No Antibiotic—No Hormone					
(1) Control	517	1.92	-	8.73	-	16.9
(2) 60 μg. Reserpine	519	1.87	(-)3.0	8.92	(-)2.5	17.5
(3) 25 mg. Rauwolfia	520	1.94	0.0	9.06	(-)3.8	17.4
(4) 1.25 mg. Tran-Q	523	1.85	(-)3.7	8.92	(-)2.2	17.3
(5) 2.50 mg. Tran-Q	528	1.84	(-)4.0	9.23	(-)5.7	17.9
(6) 5.00 mg. Tran-Q	527	1.93	0.0	8.69	0.7	16.8
Av. Lots (1) to (6)	522	1.89	(-)1.0	8.93	(-)2.3	17.3
	Stilbestrol Implant (36 mg.) + Terramycin (80 mg.)					
(7) Control	521	2.14	-	8.83	-	17.1
(8) 60 μg. Reserpine	521	2.28	6.7	8.55	3.2	16.5
(9) 25 mg. Rauwolfia	515	2.27	6.0	8.63	2.3	16.6
(10) 1.25 mg. Tran-Q	525	2.25	5.0	8.53	3.5	16.5
(11) 2.50 mg. Tran-Q	516	2.53*	18.0	8.14	7.8	16.0
(12) 5.00 mg. Tran-Q	523	2.18	2.0	8.72	1.2	16.9
Av. Lots (7) to (12)	520	2.28	6.0	8.57	3.0	16.6

*Difference in daily gain is significant at 5 per cent level.

Results with Tran-Q in cattle feeding are summarized in Table 10.

TABLE 10

Summary of Steer Feeding Trials with Tran-Q

(Other Simultaneous Treatments Indicated Thus: S = Stilbestrol Implant; TMS = Stilbestrol Implant and Oral Terramycin)

Source*	Contr.	Trtd.	Tran-Q Mg./Hd./ Day	Growth Incr. (%)	F. E. Incr. (%)	Carcass Grade Diff. from Contr.	Ret. over Contr./ Animal ($)
16.1-19	16	8	1.25	6.1	5.2	+0.63	12.72
16.1-16; 15.3-48 S	5	4	1.25	26.0	12.1	(-)0.20	12.51
Purdue	6	6	1.25	(-)3.7	(-)2.2	-	-
Purdue TMS	6	6	1.25	5.0	3.4	-	-
15.3-29 TMS	5	4	2.4	9.0	5.0	+0.40	18.04
16.1-11; 15.3-35†	16	8	2.4	5.0	4.0	(-)0.06	(-)0.11
16.1-11; 15.3-35 TMS†	16	8	2.4	2.0	0.0	(-)0.16	0.68
16.1-16; 15.3-48 S	5	4	2.5	19.0	12.0	+0.80	17.01
Purdue	6	6	2.5	(-)4.0	(-)5.7	-	-
Purdue TMS	6	6	2.5	18.0	7.8	-	-
Mississippi	6	6	5.0	0.0	19.0	(-)0.67	-
Purdue	6	6	5.0	0.0	0.7	-	-
Purdue TMS	6	6	5.0	2.0	1.2	-	-
Oklahoma S	10	10	‡	4.0	5.0	-	5.00
Mississippi	6	6	10.0	14.0	23.6	+0.34	-
16.1-19	16	8	10.0	4.6	1.0	(-)0.13	3.68
16.1-16; 15.3-48 S	5	4	10.0	25.0	12.0	(-)0.20	7.31
16.1-16; 15.3-48 S	5	4	50.0	22.0	16.7	+0.10	10.86
16.1-16; 15.3-48 S	5	4	500.0	16.0	12.0	(-)0.10	(-)8.47
Averages 1.25 mg. to 10 mg.				7.7	6.1	+0.07	$8.55

*Numbers designate Pfizer studies.

†This trial reported in full in a paper now in press.

‡1 mg. daily to 90 days; 5 mg. thereafter.

A consistent relationship of level to growth response is not indicated. A positive effect was shown in thirteen of seventeen growth-rate comparisons and the same for feed efficiency. There are not enough data to evaluate adequately whether or not a synergism exists among Tran-Q, stilbestrol implants, and Terramycin. The Purdue results appear to indicate it, but, in another study (Pfizer 16.1-11; 15.3-35), the growth and feed efficiency increments were greater percentage-wise in the absence of the other agents.

For the seventeen trials at Tran-Q levels up to and including 10 mg. per day, the average growth increment over controls, combining trials with and without other stimulants, was 7.7 per cent, and the feed efficiency increment was 6.1 per cent. There was no indication of an effect on carcass grades. To date, however, the negligible changes in average carcass grade as a result of treatment with tranquilizers, as well as shrinkage data which indicate no difference between treated and controls, are indirect evidence that carcass composition is essentially unaffected by the feeding of a tranquilizer such as Tran-Q.

Economic data where available within the dose bracket under consideration showed returns over controls varying up to $18.04 per animal, average

being $8.55.[3] It would appear that substantial results can be expected in the 2.0 to 5.0 mg. range.

MECHANISM OF GROWTH PROMOTION

Reasons for the growth-stimulating action of tranquilizers are not yet apparent. The obvious surmise that the drugs reduce physical activity and thus diminish energy expenditure was not sustained by frequent observations of the activity of the animals in the Pfizer trials. These observations also showed no gross evidence that any of the levels used for steers and lambs brought about sedation. It is possible that small doses of tranquilizing drugs exert a protective effect against environmental stress which does not present itself in the animals' gross behavior or otherwise. Conceivably also the action is mediated through the nervous system to the organs of digestion and assimilation.

SAFETY CONSIDERATIONS

Toxicity tests on Tran-Q have been conducted with a variety of animal species. For purposes of this paper, only the information pertaining to ruminants will be mentioned.

Cattle: Tran-Q fed to 1,000-lb. animals in the amounts of 1-9 gm. for 7 days elicited no adverse side effects (no tranquilization). This drug was fed also to steers at a rate of 0.5 gm. per day for 112 days. No histopathology was found in liver and kidney tissues.

Lambs: Tran-Q has been fed to lambs continuously for 69 days at levels of 45 mg. per day with no evidence of side effects. Still larger doses were tried for periods of 4 days. Tranquilization and other effects appeared only at levels as high as 2,330 mg. per animal. No signs of gross pathology were observed in tissues of lambs fed Tran-Q at 45 mg. per day for 69 days.

SUMMARY

Feeding trials available to date on the use of certain tranquilizers as growth stimulants for wether lambs and steers have been reviewed. These show that one compound, Tran-Q, within what seems practical dosage limits, brought about substantial increases in rate of gain and efficiency of feed conversion in both species and did not affect carcass quality. In several comparisons, improvement in performance with this tranquilizer was superimposed upon increments obtained with Terramycin or stilbestrol, or both. A financial advantage from the use of this tranquilizer is indicated in the average results of these trials. The safety of feeding Tran-Q has been demonstrated in several species in which levels were tested far in excess of those required to increase growth. The mechanism whereby tranquilizers enhance productive performance of ruminants is not yet known. Levels which promote growth do not induce gross signs of sedation, nor do they diminish physical activity of the animals.

3. In the Pfizer trials, feed costs were based upon prices in Chicago.

X

ANALYTICAL VARIATIONS IN SAUSAGE COMPOSITION

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This presentation is a summary of a series of studies undertaken by the Technical Advisory Committee on Moisture in Sausage, which was a part of the Sausage Committee of the American Meat Institute. This Advisory Committee was asked to study the problem of moisture control and regulation in cooked-sausage items. Previous reports discussed some aspects of the economics of producing sausage in compliance with the Meat Inspection Division, USDA, regulation that moisture must not exceed four times the protein plus 10 (M = 4P + 10).

There has been a desire for some time to modify this regulation because of the fact that analytical protein, and hence analytical error, is multiplied fourfold in determining moisture limits.

This report covers three separate studies: (1) the interchangeability of moisture and fat; (2) the magnitude of errors in the analysis of sausage for protein; and (3) the errors inherent in sampling and subsequent analysis for moisture and protein.

The first phase was undertaken in an effort to determine whether the protein-moisture ratio in sausage manufacture was sufficiently rigid to permit the substitution of the formula "moisture plus fat equals 85 per cent or less (M + F = 85 per cent or less)" for the standard M = 4P + 10. In addition to the moisture-plus-fat limits, it was understood that additives would not exceed 5 per cent, and hence a minimum protein content of 10 per cent would be assured. Under present regulations no minimum protein level is called for. It was foreseen that the Meat Inspection Division might object to this proposal unless it were demonstrated that protein content limited moisture content within the same relative limits as found in the current regulation.

Ten companies participated in this study. They were asked to produce all-meat frankfurters and bologna at high- and low-protein levels according to their own formulas but with the specification that three levels of moisture addition be observed: (a) normal amount of ice; (b) maximum acceptable amount for branded product; and (c) maximum amount which could be incorporated into the sausage emulsion without seriously affecting quality.

It was found that, although moisture could be used in quantities greater than those permitted under the formula 4P + 10, the total amount appeared to be limited by the amount of protein present. However, the data were not sufficiently extensive to determine the absolute limit in moisture content.

The results obtained in this study are shown summarized and tabulated in Table 1. In the case of the "high-protein" frankfurter and bologna, some of the product made with maximum possible moisture added exceeded the 4P + 10 limits by approximately 10 per cent. Table 1a shows the average totals of fat plus moisture and indicates that a regulation M + F = 86 per cent would cover all cases encountered in this study. However, the interchangeability of fat and moisture, even to the extent indicated in this study, caused the Advisory

TABLE 1

Statistics from American Meat Institute Sausage Committee Study

	Low Protein			High Protein		
	Normal	Max. Acc.	Maximum	Normal	Max. Acc.	Maximum
Franks:						
Mean H_2O ± 95% limits	52.10 ± 6.42	52.96 ± 5.92	54.15 ± 5.84	57.07 ± 8.24	59.23 ± 7.62	60.26 ± 10.00
Mean protein ± 95% limits	11.37 ± 2.22	11.08 ± 2.58	10.74 ± 2.26	12.46 ± 2.26	12.36 ± 0.56	11.53 ± 1.18
Bologna:						
Mean H_2O ± 95% limits	52.68 ± 8.56	53.76 ± 8.54	55.34 ± 8.18	55.93 ± 5.40	60.32 ± 7.56	62.76 ± 8.78
Mean protein ± 95% limits	10.81 ± 2.00	10.50 ± 1.70	10.33 ± 2.34	12.46 ± 1.60	11.81 ± 2.08	11.57 ± 1.80

TABLE 1a

Mean Moisture Values—Compliance with M + F = 86

(Additives Assumed = 4 Per Cent)

Water/Protein	Normal		Maximum Acceptable		Maximum Possible	
	Low	High	Low	High	Low	High
Franks	84.6	83.5	84.9	83.6	85.3	84.5
Bologna	85.2	83.5	85.5	84.2	85.7	84.3

Committee to seek elsewhere for a solution to the problem of satisfactory moisture content and control.

It was suggested that a study be undertaken which would determine the magnitude of the errors involved in protein determination and that the Meat Inspection Division be approached with the possibility of taking these analytical errors into consideration in enforcing compliance with the 4P + 10 regulation. Accordingly, a study was set up which included both industry and Meat Inspection Division laboratories and which was designed to determine the analytical errors inherent in protein analysis.

Two samples of all-meat bologna were produced which had very similar protein content. These samples were comminuted and blended in such a way that they differed from each other in protein content by less than 1 per cent. Samples of this well-blended product were sent out to approximately fifty-seven industry and Meat Inspection Division laboratories in tightly stoppered glass sample jars. The laboratories were instructed in the manner of sampling and in the number and kind of analyses to be performed. It was requested that at least two chemists in any one laboratory perform the analyses, in triplicate, on each of two successive days. Samples were to be taken from the sample jars after the surface layer of meat had been skimmed off. These were to be grab samples, and standard procedures used for protein analysis by the laboratory were to be followed. The data sheet which was returned to the American Meat Institute Foundation requested that each respondent list the approximate sample weight, the catalyst used, the digestion time in hours, and the normality of the standard acid and base. The protein was to be reported on the basis of nitrogen times 6.25 to the nearest one-tenth of 1 per cent.

As might be expected, the catalysts used varied from company to company and included ten variations. The digestion time varied from 1 to 3 hours, and the normality of the acid ranged from 0.1 to 0.5. The normality of the base also ranged from 0.1 to 0.5. The individual reported analytical values for the first of the two samples ranged from 11.8 to 13.3 per cent protein and for the second sample from 12.0 to 13.6 per cent. The mean values came out to be 12.6 per cent protein for the first sample and 12.8 per cent protein for the second sample.

The results of these studies were analyzed by Dr. H. P. Andrews, of Swift & Company, and the following conclusions made: It was found that within 95 per cent probability limits the duplicate analysis on the same day, by the same chemist, should not differ by more than 0.38 per cent protein; that single analyses on different days by the same chemist should not differ by more than 0.40 per cent protein; that two separate chemists within a laboratory should not differ by more than 0.46 per cent protein; that two different laboratories performing a single analysis should differ by no more than 0.62 per cent protein. The standard deviation for any single analysis by any one chemist was 0.22 per cent. This means that an analysis should fall within $\pm$ 0.44 per cent of the average protein value 95 per cent of the time. These results are tabulated in Table 2.

Taking into account this standard deviation and standard error for protein analysis, it can be seen that, if such an error is multiplied by 4 (in the formula 4P + 10), we can expect that at times the moisture limit in sausage can be up to 1.6 per cent in error. This means that, based on the error inherent in protein analysis, the current regulation should be $4P + 10 \pm 1.6 = M$.

With the variation in analyses for protein fairly well established, the Advisory Committee next suggested that a study be undertaken to determine what effect the different times and places of selecting samples would have on the protein and moisture values in sausage. This study was considered important because it was expected that considerable variation might occur in different areas of the smokehouse or at different times after processing. If this were true, it would be highly desirable to have a standardized sampling procedure

TABLE 2

Tabulation of Conclusions—Protein Analysis Study

1. Duplicate analyses on the same day by the same chemist should not differ by more than 0.38 per cent protein

2. Single analyses on different days by the same chemist should not differ by more than 0.40 per cent protein

3. Single analyses by different chemists within a laboratory should not differ by more than 0.46 per cent protein

4. Single analyses from two different laboratories should not differ by more than 0.62 per cent protein

5. Standard deviation—single analysis by one chemist—0.22 per cent protein

followed by industry and the Meat Inspection Division. Again the Meat Inspection Division very graciously offered to co-operate in the study, and a rather involved sampling and analytical plan was worked out.

First, only smokehouses holding four trees and located on one floor were used for purposes of uniformity and simplicity. Seven companies agreed to participate. Each company was matched with a Meat Inspection Division laboratory.

Immediately upon removal of the trees from the smokehouse a Meat Inspection Division inspector was asked to take the following duplicate (side-by-side) samples from the trees: from the top outside edge (positions 1 and 4 in Fig. 1); from the bottom outside edge (positions 2 and 5); and from the center (positions 3 and 6). Each sample consisted of at least twelve frankfurters or a 2-inch-thick slice of 4-inch-diameter bologna. Each sample was placed immediately in a numbered polyethylene bag and sealed or tied tightly. One sam-

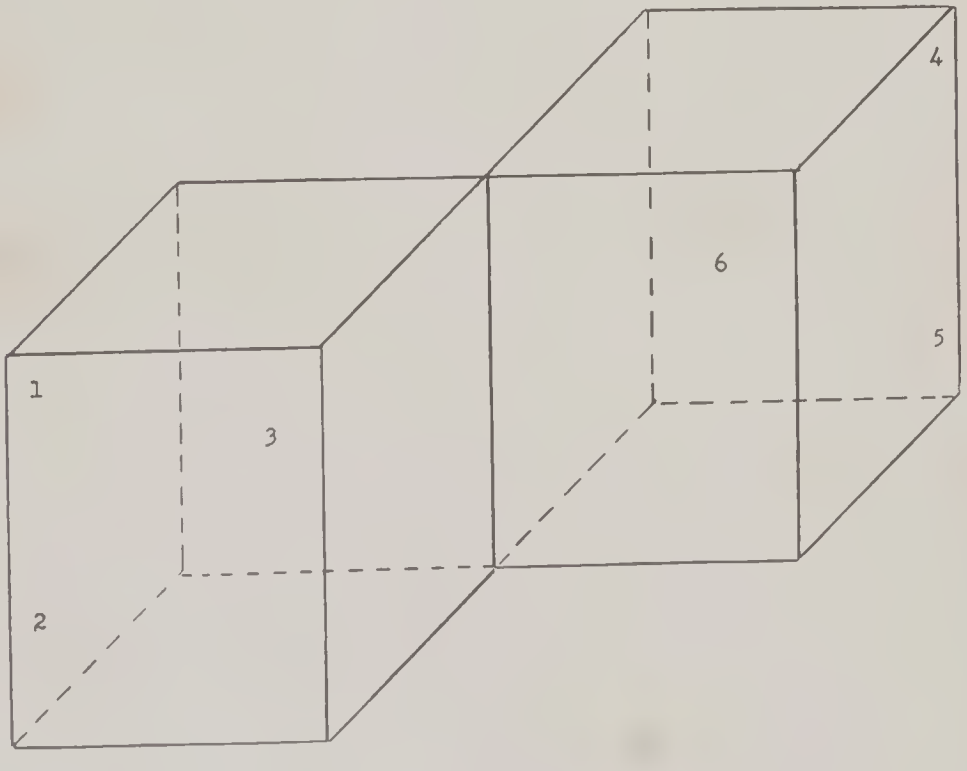

Figure 1. Sampling plan—second study

ple from each of the six sets of duplicates was sent to the company laboratory and the other to the paired Meat Inspection Division laboratory.

The trees were followed through the usual processing operation and sampled again in the cooler. At some time after 6 and before 15 hours in the cooler, another set of six duplicate samples was taken as described above, and one sample from each of the six sets of duplicates was sent to the company laboratory and the other to the corresponding Meat Inspection Division laboratory. The bologna samples were taken from the same individual bologna after a slice 1 inch thick was removed from the cut end.

Just prior to the packaging, another set of three duplicate samples was taken from either positions 1, 2, and 3 or 4, 5, and 6. One sample from each of the three sets of duplicates was sent to the company laboratory and the other to the Meat Inspection Division laboratory.

In summary, this sampling involved fifteen sets of duplicate samples from each of two lots of frankfurters and two lots of bologna or a total of sixty sets of duplicate samples. These samples were taken by Meat Inspection Division inspectors in seven plants involved in the study and forwarded to the appropriate plant and Meat Inspection Division laboratories.

The participants in this study were asked to hold the samples under refrigeration after they had arrived in the laboratory. An analysis on each lot of fifteen samples was to be completed and reported within 1 week after receiving samples. The methods of sample preparation and analysis were to be those that were used routinely in each laboratory, and each sample was to be analyzed in duplicate for moisture and protein and results reported on a standardized form. This form asked for the following information:

Moisture Analyses

The approximate sample weight
The drying time in hours
The drying temperature in degrees centigrade
The vacuum, if any, in inches of mercury

Protein Analyses

The approximate sample weight in grams
The catalyst used
The digestion time in hours
The normality of the standard acid and base

The variation in procedures for protein analysis were repeated in this study. For example, the drying time, in hours, ranged from 3 to 18, and the drying temperature ranged from 101° to 126°C. In the case of protein analysis, six different catalysts were employed, and the digestion time ranged from 45 minutes to 3 hours, and the normality of the acid ranged from 0.1 to 0.5. The normality of the base again ranged from 0.1 to 0.5.

The results of this study were subjected to extensive statistical analysis to obtain some estimates of the variability in sampling and analyses by Dr. Andrews, of Swift & Company. I quote from his report:

"In general, the observations concerning variability trends were quite similar for moisture and protein and can therefore be discussed together. The major source of variation for both moisture and protein was among the different companies, with ranges as shown in Table 3.

"Average changes in moisture and protein content during the interval from smokehouse to cooler to finished product were not statistically significant, since they were highly inconsistent from one company to another. Furthermore, they were not the same for franks and bologna.

"Positional variations within the batch were not significant, that is, differences between top and bottom or inside and outside samples were neither great

TABLE 3

Ranges—Protein and Moisture—Sampling Study

	Out of Smokehouse	In Cooler	Finished Product
Per cent protein:			
Franks	10.47 - 12.14	10.53 - 12.25	10.65 - 12.31
Bologna	10.22 - 12.07	10.18 - 12.21	10.15 - 12.24
Per cent moisture:			
Franks	51.36 - 54.94	50.16 - 54.67	49.72 - 54.22
Bologna	45.49 - 56.80	45.93 - 56.67	46.38 - 56.68

enough, nor consistent enough, to be considered statistically significant. Since no patterns of variability could be established, it must be considered as random error, and it was found to be quite different in the different companies.

"Errors greater than those associated with within batch handling variations were those which were due to the inability of the Meat Inspection Division and company laboratories to obtain the same results on duplicate samples. This was shown quite clearly in the summary of the all-company mean squares shown in Table 6. Irrespective of the location from which the sampling was done, the between-laboratories variation was greater than the within-batch variation. The lack of agreement in analytical results varied from very slight agreement for some companies to that of gross disagreement for others.

"In most instances the company laboratory obtained results which were consistently higher or lower than those obtained in the co-operating Meat Inspection Division laboratory. The results were sometimes higher and sometimes lower, with no significant trend in any one direction. These inconsistent high or low results were obtained for both moisture and protein, but not in a manner which would explain either result. The tabulation of means as shown in Table 4 clearly shows this variability.

"Since the major source of error in sampling and analyzing franks and bo-

TABLE 4

Means of Analysis, Industry and Meat Inspection Division—Sampling Study

Packer No.	Protein		Moisture	
	Co. Lab.	M.I.D. Lab.	Co. Lab.	M.I.D. Lab.
1-2	11.66	11.77*	53.48*	52.41
3-4	10.82	11.27*	54.00	53.85
5-6	10.72	10.79	49.39	49.95*
7-8	11.11	11.21	51.21	51.93*
9-10	11.28	11.14	52.42	51.99
11-12	12.17*	11.53	54.36*	54.03
13-14	11.73	11.93*	55.62	55.37

*Significantly higher than corresponding laboratory.

logna for moisture and protein appears to be associated with between-laboratory variation, Table 5 shows the 95 per cent probability limit for the differences between various analytical results.

TABLE 5

95 Per Cent Probability Limits—Sampling Study

	Moisture		Protein	
	Bologna	Franks	Bologna	Franks
Duplicates in a laboratory	0.47	0.46	0.28	0.27
Single determinations, different laboratories	1.68	1.61	0.91	0.70

"From these results we would not expect duplicate protein determinations on the bologna sample within a laboratory to differ by more than approximately 0.28 per cent, and we would not expect single protein determinations on a bologna sample in two different laboratories to differ by more than 0.91 per cent, five times out of a hundred.

"It is of particular interest to note that these estimates of protein analytical error are of the same general magnitude as those obtained in an earlier study aimed at establishing the among-laboratories variation. The previous study gave an over-all standard deviation of 0.22 per cent as compared to 0.29 per cent obtained in this study. Logically, we would have expected the error to be somewhat greater in this study, since a slight, very probably real, variation within the split sample in this work was scrupulously avoided in the previous

TABLE 6

Summary of All-Company Mean Squares—Sampling Study

	Moisture		Protein	
	Bologna	Franks	Bologna	Franks
Smokehouse:				
Batches	27.7642	4.3657	0.9914	1.5086
Within batch	0.4299	0.6187	0.1134	0.0621
Laboratories in samples	1.0932	0.6216	0.1948	0.1405
Duplicates	0.0351	0.0291	0.0096	0.0094
In cooler:				
Batches	30.3029	8.9371	1.3228	1.0900
Within batch	0.4574	0.5797	0.0874	0.0654
Laboratories in samples	0.4784	0.6082	0.1661	0.1001
Duplicates	0.0233	0.0249	0.0102	0.0086
Before packaging:				
Batches	18.7985	3.5514	0.9085	0.6871
Within batch	0.3282	0.5506	0.0321	0.0857
Laboratories in samples	0.7903	0.6604	0.2610	0.0852
Duplicates	0.0200	0.0253	0.0100	0.0112

study by a careful comminution of the entire sample material before sending aliquots to the various laboratories.

"The conclusion remains obvious that present analytical practices, as carried out in various laboratories, account for the major source of discrepancies in the composition testing of sausage products. The experience in seven different packing plants in replicated lots of franks and bologna, sampled objectively according to a plan designed to detect most likely differences in product, failed to show any appreciable within-batch sampling errors."

As a result of this last study the Committee on Analytical Methods of the American Meat Institute Foundation has been requested to study a possible standardization of moisture and protein analytical procedures so that these discrepancies between laboratories can be eliminated.

ACKNOWLEDGMENT

The members of the Technical Advisory Committee on Moisture in Sausage are grateful to the American Meat Institute Foundation for the unstinting co-operation and help which its staff gave in preparing and receiving the questionnaires and samples. In particular we wish to thank Dr. D. M. Doty for the time and effort which he devoted in this direction. Further, the excellent co-operation which we enjoyed with Mr. H. P. Andrews, statistician in the Research Laboratories of Swift & Company, cannot go unmentioned, since it is largely through his efforts that we have been able to present the conclusions reported here in an interpretable manner. The wholehearted co-operation and encouragement of officials of the Meat Inspection Division is also gratefully acknowledged.

XI

MEAT TENDERIZING BY ENZYMES

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Centuries before the mechanisms of enzymatic meat tenderization were closely scrutinized by scientists, the natives of tropical and subtropical areas around the world were quite familiar with the practical application. Early accounts of such practices indicate that tenderization was accomplished by boiling the meat with unripe papaya fruit or by wrapping the meat with papaya leaves. The first scientific investigation of this application was made in 1878 by Wittmack (1). From about 1940 up to the present time, enzymatic meat tenderizers have made their appearance in ever increasing numbers. Until 1955 such formulations were available to outlets such as grocery stores, restaurants, hotels, hotel-restaurant supply houses, and small meat packers not under M.I.D. (Meat Inspection Division, USDA) jurisdiction. In June, 1955, the M.I.D.-inspected meat packers were permitted to use enzymatic meat tenderizers, and a recent report (2) indicates that they are now using over 20,000 gallons per month. The objective of such usage, of course, is to increase the tenderness of lower grades of beef to the extent of making them more acceptable for public consumption. From a nutritional standpoint, the importance of making available to the public a significantly increased quantity of high-quality protein cannot be overemphasized. Such a goal would justify beyond question any of the research that has been or is being conducted toward this end.

Commercial tenderizers consist, in general, of a small quantity of proteolytic enzyme which is either dissolved in a liquid vehicle such as hydrolyzed vegetable protein or dispersed in a dry preparation of various salts with or without seasoning. The optimum level of an enzyme preparation in a commercial tenderizer is determined by organoleptic evaluation of treated steaks. As a quality-control measure, this effective level of the particular enzyme preparation is standardized on an activity basis from one commercial production to the next by one or more proteolytic assay methods.

Meat-tenderization studies have brought to light the fact that, in addition to papain, the most commonly used enzyme in commercial preparations, other proteolytic enzymes of plant, animal, and microbiological origin are also effective in this regard. The use of bromelain as a meat tenderizer was suggested by Balls (3) in 1941. At the present time commercial meat tenderizers are being used in which papain, bromelain, ficin, and a fungal enzyme, Rhozyme P-11, respectively, constitute an active ingredient.

The diverse origins of the proteolytic enzymes merit a brief description at this point. The sources of plant proteases are illustrated (at the harvesting stage) in Figures 1 and 2. Papain (8,9) is the dried latex of the fruit of the tree Carica papaya (Fig. 1), which is cultivated extensively in Ceylon and in British East Africa. The fig tree of the genus Ficus is the source of fig latex (Fig. 2) from which the enzyme ficin (10) is isolated. It is grown in Central and South America. Ficin is imported mainly from South America. Bromelain (11) is commercially produced from the stem of the pineapple, Ananas comosus (Fig. 3), and is imported from the Hawaiian Islands. Bacterial protease can be obtained by the culture of Bacillus subtilis. For the commercial production of

Figure 1. Commercial papain is derived from the latex which exudes from the green fruit of the papaw tree, Carica papaya.

fungal protease, selected strains of Aspergillus oryzae or A. flavus are commonly used. These micro-organisms secrete enzymes which can subsequently be processed by several techniques (12). Animal proteases, such as trypsin and pepsin, are produced commercially from hog pancreas and the mucosa of hog stomachs, respectively. It should be pointed out that all these enzymes are mixtures of proteases, inert protein, and ash and, in some cases, contain amylases, oxidases, peroxidases, acid phosphatase, and other enzymes. Impurities not already mentioned may also be present in significant amounts.

With the advent of enzyme preparations from plant, animal, and microbiological origin into the field of meat-tenderization studies, the need for an investigation of the extent and mechanism of tenderization by each of these enzymes was quite evident. The problems confronting such an investigation were numerous and complex in nature. The enzyme preparations used, even if purified and standardized to uniform proteolytic activity, are still to be considered as mixtures of enzymatic and non-enzymatic constituents. Assay methods for these enzymes do not necessarily correlate with the end use for which the enzyme is intended. However, the proteolytic assays do at least form a precise

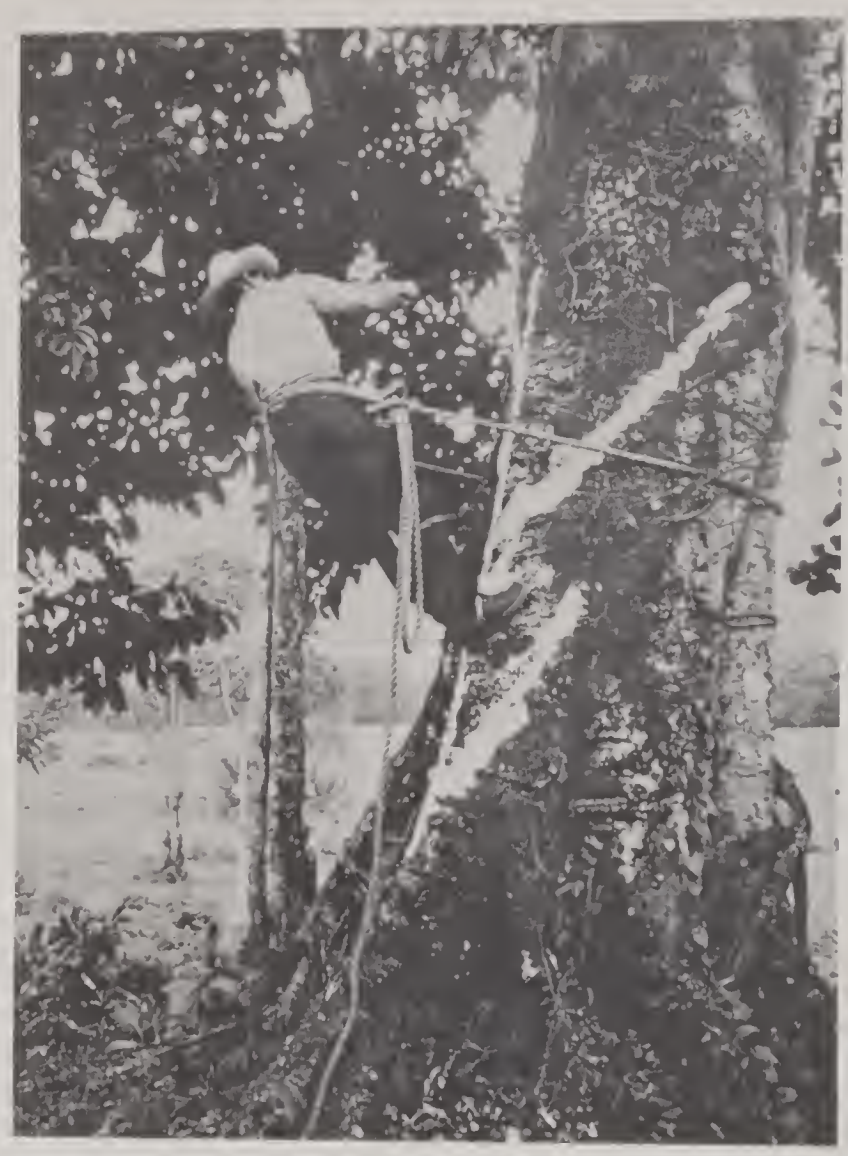

Figure 2. The fig tree of the genus Ficus is being tapped for fig latex from which ficin is isolated.

quality-control tool for manufacturing tenderizers. Meat, on the other hand, is a heterogeneous substrate composed mainly of proteins of widely differing properties. Among these are actomyosin, or muscle-fiber protein, which is a globulin by virtue of its solubility. Collagen and elastin, which together comprise the fibrous components of connective tissue, are albuminoids. Toughness in meat is associated with the collagenous and elastic components of the connective tissues. The ground substance (13) of the connective tissue in which the collagenous and elastic fibers are imbedded contains mucopolysaccharide-protein complexes and is amorphous in nature. Considering the relative specificities of various proteolytic enzymes toward different substrates and also the fact that their pH optimums do not necessarily coincide with the pH of meat, the need for screening proteolytic enzymes of different properties and origin is quite obvious.

Extensive investigation of the relative potencies of proteolytic enzymes of plant, animal, and microbiological origin as meat tenderizers has been carried out by Miyada and Tappel at the University of California (6) and by Wang et al. at the American Meat Institute Foundation (7). The problem of penetration of enzyme into raw meat, although still problematical, was substantially overcome in experimental studies by freeze-drying techniques which permit the rehydrating liquid quickly to bring the enzyme into contact with the tissue components.

The chemical approach used by Miyada and Tappel revealed differences

in the relative amount of hydrolysis of beef proteins produced by various proteolytic enzymes. Rehydrated ground, freeze-dried meat was used as the substrate. The extent of digestion of the various meat components was ascertained by separating the soluble nitrogen fraction into soluble protein nitrogen and non-protein nitrogen. The insoluble protein fraction was separated into collagen and elastin. Kjeldahl and hydroxproline determinations were made on each fraction in order to assay the degree of enzymatic hydrolysis. The proteolytic activity of various enzymes toward soluble muscle-fiber protein in order of decreasing activity was shown by ficin, bromelain, trypsin, papain, and Rhozyme P-11. Although definite concentrations of each enzyme were used, it was pointed out that no index of purity or activity of these enzymes was available. A comparison of the relative rate of hydrolysis of muscle-fiber protein by these enzymes was then made on the basis of their activity as determined by hemoglobin assay. The increase in non-protein nitrogen catalyzed by each enzyme was divided by the activity of that enzyme in the hemoglobin assay. From these results it was found that the three plant enzymes, papain, bromelain, and ficin, had approximately the same proteolytic activity and showed no pronounced differences. Of the three microbiological enzymes, Rhozyme P-11, Rhozyme A-4, and Protease 15, Rhozyme P-11 was found to be the most effective. A relatively large hydrolysis of muscle protein was exhibited by trypsin.

Elastin was digested effectively by papain and ficin and only slightly by Rhozyme P-11, Protease 15, and Rhozyme A-4. Collagenase activity was shown in order of decreasing activity by bromelain, ficin, trypsin, Rhozyme P-11, and papain.

It was concluded from this study that most of the enzymes studied, with the exception of pepsin, merit further attention as meat tenderizers. A definite recommendation for the use of any particular enzyme could not be made on the basis of this work alone. Further experimentation along these lines using whole steaks rather than ground meat and subsequent use of mechanical shear-force measurements and organoleptic evaluation techniques were suggested.

In contrast to the chemical technique of Miyada and Tappel, a histological or structural approach was used by Wang et al. to determine the extent and mechanisms of action of the various proteolytic enzymes on beef protein. Essentially, structural changes were determined from microscopic sections of fixed and stained samples of freeze-dried beef steaks after 15 minutes rehydration in aqueous enzyme solutions. Single muscle fibers or perimysial sheets containing collagen and elastin fibers obtained by a blendoring technique were also used to observe structural changes. It was found that the response of muscle fiber to proteolytic enzyme action was characterized first by disintegration of the sarcolemma and nuclei followed by the complete disappearance of the cross-striations. This, in turn, was accompanied by a gradual decrease in extensibility of the muscle fiber. The degradation of collagen by enzymes was indicated by a decrease in staining capacity with acid fuchsin as well as a gradual loss of discreteness in the fibrillar nature of collagen, the latter probably resulting from the liquefaction of the matrix, the material in which the connective-tissue fibers are imbedded. Elastin digestion was recognized in three stages: (1) zero effect if treatment with Weigert's stain indicated intensely stained, intact fibers; (2) intermediate but positive elastase activity if the fibers showed a typical pattern of segmentation when stained; and (3) complete digestion of elastin when the fibers could no longer take the stain at all. This final stage was revealed when only a trail of "ghosts" remained as an indication of the original location of the previously intact elastic fibers. The enzyme has then completely dissolved the elastin.

From these structural criteria, it was concluded that the proteolytic enzymes of microbiological origin as a group exhibited potent activity toward muscle fiber and in some cases slight activity toward collagenous fibers. Ficin and papain, as well as the pancreatic enzymes, trypsin and Viocase, also showe

considerable activity toward muscle fibers. Bromelain showed the least relative potency in this regard. The plant enzymes as a group showed potent activity toward the components of connective tissue. Trypsin and Viocase showed slight activity in this respect. The microbiological enzymes exhibited no elastase activity at all and, as mentioned previously, only traces of collagenase activity in some cases.

Several of the proteolytic enzymes used in the histological study were evaluated organoleptically. It was found that, when whole freeze-dried steaks were rehydrated with very low concentrations of either a plant or a fungal protease, the taste panel detected definite increases in tenderness when compared with steaks which were rehydrated in water or a 2 per cent sodium chloride solution. The addition of 2 per cent sodium chloride to the rehydrating water in itself increased tenderness. When salt and enzymes were added together, the effect was additive. The main tenderizing effects of the enzymes occurred at temperatures between 40°F. and the final cooking temperature of 150°F.

Subsequent correlation of organoleptic and structural criteria of tenderness indicated that the organoleptic threshold for determining increases in tenderness was by far the most sensitive. According to Wang et al., this reflected only a differential sensitivity to the two kinds of criteria studied and not a lack of correlation between tenderness and structural changes caused by enzymatic treatment. If Wang's inference is correct, this comparative lack in sensitivity is more than compensated for by the substantial contributions made by these histological studies toward an understanding of the mechanisms of enzyme action in increasing the tenderness of meat. It was concluded that any one of the enzyme preparations studied could tenderize meat, assuming that the proper concentration of enzymes could penetrate the tissue evenly.

In the broadest sense structural and chemical methods of assaying the tenderizing capacity of various proteolytic enzymes agree insofar as all the enzymes with the exception of pepsin were found to be effective in hydrolyzing or altering the structure of one or more components of muscle tissue. However, when an attempt is made to correlate the relative effectiveness of each enzyme regarding the mechanism of action and extent of hydrolysis, a considerable amount of disagreement can be noted. Whereas the structural studies indicated that bromelain contained only a trace of activity toward muscle fiber, the criteria used in the chemical approach described it as potent in this respect. The fungal and bacterial proteases showed no elastase activity by structural criteria but did show slight activity toward elastic fibers when evaluated by chemical methods. Finally, collagenase activity indicated by the fungal enzyme Rhozyme P-11 in chemical studies was not indicated by structural criteria.

When one considers the diversity of conditions encountered in the assay of tenderness by structural and chemical methods, it is not in the least surprising that such discrepancies appear. Inherent error in the methods themselves should be considered. It was reported by Neuman and Tytell (14), for example, that the sensitivity of collagens to attack by proteolytic enzymes is affected by prior treatment. In any enzyme reaction, conditions such as pH, temperature, reaction time, the activity of the particular enzyme preparation, and the physical state of the substrate are all important in determining the resultant activity of the enzyme during the course of the reaction. Since these conditions were not intended to be the same in the structural and chemical studies, one would certainly expect differences to exist in any such evaluation of proteolytic enzymes relative to one another.

In the chemical and structural studies which have just been reviewed, all investigation of the connective tissues was concerned solely with the fibrous constituents. Little or no attention was given to an investigation of the mucinous adhesive ground substance. Miller and Kastelic (13) attempted to account quantitatively for the total nitrogen content of muscle tissue. It was demon-

strated that from 80 to 85 per cent of the total nitrogen of muscle tissue was extracted with potassium chloride solution. Assuming that only the connective-tissue fibers contain hydroxproline, 5-6 per cent of the remaining nitrogen was characterized as collagen and elastin. It was postulated that the fraction which does not contain hydroxproline and which can be solubilized by autoclaving and by alkali may be an integral portion of the ground substance. Their studies of papain lent credence to the theory proposed by Day (15) that the action of certain proteases resulted in the cleavage of linkages which were postulated to serve as scaffolding of the continuum of the ground substance.

Gottschall and Kies (4) in 1942 and Tappel (16) in 1955 found that there was no apparent advantage in preholding meat treated with papain before cooking. Evidence obtained from these studies, as well as from others, indicates that enzymatic tenderization of meat takes place for the most part during the cooking process. It has also been pointed out that physical means of incorporating the enzyme preparation into the meat prior to cooking enhances considerably the tenderizing effect. Tappel has shown that effective penetration of papain into meat cannot be accomplished by natural diffusion or by hydrolytic action of papain. Numerous techniques have been described in patents for injecting enzymes into the muscle tissue or vascular system of either chilled or freshly killed, warm carcasses (17). One of the meat packers reportedly (2) has been investigating the feasibility of injecting proteolytic enzymes directly into live cattle just prior to slaughter, thereby distributing the enzymes evenly in the meat by way of the circulatory system. A typical procedure used by meat packers in tenderizing steaks is to dip the steaks in a solution of the tenderizer for approximately 1 minute and then freeze them to arrest the action of the enzyme. It has been observed that the best effects are obtained when the dipped steaks are pressed momentarily against a plate supporting numerous small needles.

After a careful examination of the research done on the evaluation of proteolytic enzymes as meat tenderizers, several important problems became apparent. The organoleptic techniques for evaluation of the tenderizing effect of enzymes, although by far the most sensitive to desirable changes in tenderness, do not reveal as much as the structural or chemical techniques regarding the mode of attack by these enzymes. The structural and chemical techniques, on the other hand, are much more informative concerning the mechanisms of enzyme action but require, unfortunately, that the muscle proteins undergo extensive hydrolysis in order to produce a measurable change. Such changes by organoleptic criteria would be equivalent to overtenderization. The possibility that the changes in muscle tissue brought about by extensive hydrolysis cannot be extrapolated back to desirable increases in tenderness noted in organoleptic taste-panel work should be considered. In this respect, more evidence is needed to indicate whether or not a definite correlation exists between organoleptic and chemical or structural studies.

Any future research done on ascertaining the correlation between chemical and structural or other studies should include, wherever possible, an exacting reproduction of experimental conditions such as pH, reaction time, temperature, enzyme activity, etc. Furthermore, an evaluation of enzyme assay methods relative to their correlation with methods of assaying tenderness in meat would probably also be helpful in eliminating some of the problems confronting workers in this field. This point is especially important as a quality-control problem when one considers the fact that such low levels of enzyme activity are required in tenderizers. As pointed out by organoleptic studies at the American Meat Institute Foundation (7), overtenderization is worse than no tenderization at all.

In spite of the fact that a certain amount of disagreement was noted in comparing the relative effectiveness of the various enzymes as meat tenderizers, these studies do encourage speculation regarding a combination of proteolytic

Figure 3. A demonstration of a stump harvester used for harvesting the raw material for commercial bromelain production. (Dole Photo.)

enzymes that might function more efficiently in a tenderizing formula than any single enzyme. It must be pointed out, however, that combinations of proteolytic enzymes should not be made solely on the basis of their individual effectiveness as tenderizers. Research done at our laboratories indicates that certain combinations of proteolytic enzymes are not compatible in solution due to varying degrees of mutual digestion and/or inhibition. The results of this work have not as yet been published.

An investigation of the stability of proteolytic enzymes at a series of temperatures including cooking temperatures might prove valuable from the standpoint of obtaining information on time and temperature of inactivation during cooking and also on the shelf life of the commercial tenderizer. In addition, the cost of each enzyme per unit of activity as well as its availability are also important factors. Regarding methods of assaying tenderness, further research is needed for the development of techniques that not only could approximate the sensitivity of organoleptic methods but also could further the existing knowledge regarding the changes that occur when meat is tenderized.

ACKNOWLEDGMENT

The author wishes to thank R. M. Heinicke, of the Pineapple Research Institute; J. R. Wagner, of the Chemical Division of Merck & Co., Inc.; and M. D. Labbee, of Rohm & Haas Co., for pertinent unpublished information concerning bromelain, ficin, and Rhozyme P-11, respectively.

The assistance of H. Wang, now at Stritch School of Medicine of Loyola University, and Mr. Lee Burt, of Hercules Powder Company, for reading the preliminary manuscript and offering helpful suggestions is gratefully acknowledged.

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XII

INFLUENCE OF SIRES ON TENDERNESS OF BEEF[1]

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The major problem concerning beef quality is probably that of tenderness. The high degree of variability in tenderness and the frequency of toughness often perplex the consumer and create a definite problem. The meat industry has shown considerable interest in this problem, and research concerning tenderizing and associated problems has been active and apparently is increasing in intensity. The producer has shown less interest, perhaps because he is removed one or more steps from the consumer. Nonetheless, it is also his problem in that his market is affected by a direct chain of economic links. At the producer level three basic approaches are available: nutrition, management, and breeding.

The Texas Station has a project designed to investigate the breeding or inheritance phase of these three interrelated approaches. The objective is to determine sound criteria for selective breeding of beef cattle for improvement in economic traits. Among the various aspects being considered from a long-range point of view is quality of the final product. The following progress report of this work is limited to the consideration of tenderness.

MATERIALS AND METHODS

The cattle used in this study were yearling steers raised at Substation 23 and slaughtered at the meats laboratory at A. & M. College in 1954, 1955, and 1956. The steers were born in the late winter and early spring and were run with their dams without supplemental feeding until fall, when they were placed in feed lots for a gain-performance test of approximately 5 months' duration. In 1954 one-half of the steers were fed a high-concentrate ration and the remainder a low-concentrate ration, while in the other two years all steers received the high-concentrate ration. At slaughter the average age of steers was 14-15 months and the average weight approximately 800 pounds. Grades placed on the carcasses by the area supervisor of USDA federal meat-grading service ranged from utility to prime and averaged good.

The carcasses were aged at about 36°F. for 7 days, after which time various carcass traits were measured. Loin and round steaks, cut at this time, were wrapped individually, frozen at -20°F., and stored at 0°F. until tests were made. Broiling and braising were the methods used in cooking each test series for the loin and round of each animal. In 1954 the meat was all cooked well done, but in the following years it was cooked both well done and rare. Cooking test conditions were standardized as reported by Cover et al. (4, 5).

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1. Part of this work is co-operative with the U.S. Department of Agriculture and is part of the S-10 Southern Regional Beef Cattle Breeding Project.

2. The senior author is located at Substation 23, McGregor, Texas.

TABLE 1

Average Shear-Force Values for Sires

Sire Code	Sire Breed	No. of Progeny	Av. Pounds Shear Force*	Breeding of Progeny†
7	Hereford	4	8.38	He
27	Hereford	6	7.95	He
89	Hereford	4	8.70	He
90	Hereford	8	8.21	He (7), 3/4 He - 1/4 B (1)
134	Hereford	4	9.20	He (2), 3/4 He - 1/4 B (2)
136	Hereford	6	8.10	He (4), 3/4 He - 1/4 B (2)
257	Hereford	4	9.33	He (2), 3/4 He - 1/4 B (2)
259	Hereford	3	8.63	He (2), 3/4 He - 1/4 B (1)
113	Brahman	4	10.00	F_1, B x He
115	Brahman	4	7.68	F_1, B x He
117	Brahman	4	8.48	F_1, B x He
119	Brahman	4	10.93	F_1, B x He
120	Brahman	11	9.30	B (1); F_1, B x He (8); 3/4 B - 1/4 He (2)
121	Brahman	6	10.08	B (1); F_1, B x He (2); 3/4 B - 1/4 He (3)
139	Brahman	9	10.99	B (3); F_1, B x He (4); 3/4 B - 1/4 He (2)
254	Brahman	6	8.73	B (2); F_1, B x He (2); 3/4 B - 1/4 He (2)
255	Brahman	5	8.96	B (1); F_1, B x He (2); 3/4 B - 1/4 He (2)
256	Brahman	5	10.52	B (2); F_1, B x He (2); 3/4 B - 1/4 He (1)
122	Santa Gertrudis	6	9.18	S.G. (4); F_1, S.G. x He (1); 1/2 S.G. - 1/4 He - 1/4 B (1)
123	Santa Gertrudis	10	8.36	S.G. (7); F_1, S.G. x He (1); 1/2 S.G. - 1/4 He - 1/4 B (2)
137	Red Poll	8	8.83	F_1, R.P. x He (4); 1/2 R.P. - 1/4 He - 1/4 B (4)
160	Holstein	2	8.05	Ho
161	Holstein	2	8.90	Ho
90	Jersey	1	9.00	F_1, Jersey x B

*Cores were 1/2 inch in diameter. Values up to 8.5 were considered to be tender; 8.5 - 11.5, medium; and over 11.5, tough. In 1954 four values, all from well-done steaks, were averaged, and in 1955 and 1956 eight values, four each from well-done and rare steaks, were averaged.

†He = Hereford; B = Brahman; S.G. = Santa Gertrudis; R.P. = Red Poll; Ho = Holstein. The number in parentheses represents the number of individuals.

After the steak was cooked, an individual muscle was used for scoring by a trained panel. The muscles used were the longissimus dorsi of the loin and biceps femoris of the bottom round. Shear-force values were obtained with a Warner-Bratzler shearing device. Four cores 0.5 inch in diameter were obtained, two at one end of the steak and two at the other. The steak thickness allowed only one shear from each core, making a total of four shears from each steak.

During the three-year period, 126 steers, representing the progeny of 24 sires, have been sampled. The sires were: 8 Herefords, 10 Brahman, 2 Santa Gertrudis, 2 Holstein, 1 Red Poll, and 1 Jersey. A number of the steers were crossbreds (Table 1).

RESULTS AND DISCUSSION

An earlier report involving only the 1954 steers (Cover et al. [6]) which were Hereford and Hereford-Brahman crossbreds (F_1 from Hereford dams and Brahman sires) showed no significant difference between these breed groups in shear-force values or tenderness scores. However, sires within breeds were significantly different. Of the nine sires represented in the 1954 study, the progeny of one Brahman was least tender as measured by either scores or shears for both cuts and methods of cooking, while the progeny of another Brahman sire was most tender. Table 2 shows that there was considerably more variation among the progenies of the Brahmans than among those of the Herefords. Carpenter et al. (2) reported that steaks and roasts from animals of part-Brahman breeding were less tender than those from animals of Shorthorn breeding. It appears from the observed variation in sires that the particular sample of Brahmans used might influence breed comparisons to a considerable degree. Black et al. (1) found roasted meat from Brahman-Short-

TABLE 2

Range in Tenderness

Cut, Method of Cooking, and Breed*	Scores †			Shears ‡		
	Toughest	Tenderest	Coefficient of Variation (Per Cent)	Toughest	Tenderest	Coefficient of Variation (Per Cent)
Loin:						
Broil:						
H	5.0	8.4	15.61	12.9	7.6	15.71
B x H	2.6	9.0	25.09	14.5	7.0	18.62
Braise:						
H	5.7	9.2	13.27	13.8	6.9	19.00
B x H	3.6	9.7	29.04	15.2	6.2	25.60
Bottom round:						
Broil:						
H	6.2	8.7	8.65	9.8	7.6	14.86
B x H	3.2	8.3	38.68	11.1	7.4	25.14
Braise:						
H	7.4	9.5	15.91	7.2	4.1	20.04
B x H	3.9	8.7	25.67	9.8	4.1	40.28

*The sires were either Hereford or Brahman, but all the dams were Hereford.

†Average of ten judgments (five from each of two steaks) from one animal. The highest scores were given to the most tender meat.

‡Average of eight shears (four from each of two steaks) from one animal. The least tender meat required the most force to shear.

horns and concluded, since wide individual variation was observed, that possibilities for improvement by selection existed.

The extent to which hereditary differences are responsible for the observed differences in the tenderness of the meat of one steer as compared to that of another can be measured by heritability estimates. From the 1954 steers, heritability estimates for different cuts and methods of cooking ranged from 28 to over 100 per cent for tenderness scores and from 85 to over 100 per cent for shears. Such values, owing to the method of calculation, are subject to large errors, and some are obviously overestimates, as 100 per cent is the upper limit. Heritability estimates apply directly only to the group sampled, but more general implications are warranted as samples become larger and more representative. The above values, at most, indicate that the heritability of tenderness is probably high or moderately high. If further research by several workers substantiated values even as low as 30-40 per cent, it would be known that considerable progress in changing the particular trait could be made by rather simple selection. Hiner and Yao (7) reported no data but stated that "resistance to shearing" was highly heritable.

Tenderness of beef is a complicated trait because muscle tissue and connective tissue are both involved. It has been observed that in one cut of meat the connective tissue was tender while the muscle tissue was tough and vice versa. Muscle-tissue quality can be described more exactly by the degree of friability and softness rather than by tenderness alone. Steaks from the steers slaughtered in 1956 were scored for friability, softness, and amount of residual connective tissue and were sheared mechanically (Cartwright et al. [3]). Loins and bottom rounds were broiled to internal temperatures of 61° and 80°C., respectively, and the two cuts were braised to an internal temperature of 85°C. and 100°C., respectively. Of the eight possible temperature-cut categories, no significant difference between breeds was found for residual connective tissue, but for softness, friability, and shears significant differences were found in several of the categories. For sires within breeds several categories showed significant differences with respect to all traits. These results, although still indicating that inheritance does affect tenderness, were desultory and fit no obvious pattern.

In contrast to investigating each aspect of tenderness, an over-all average estimate of tenderness may be used. Certainly, the varied consumers in America have different tastes, buy different cuts, and use an almost infinite number of temperature-method-cut combinations in cooking beef. For practical application in evaluating sires an average of several values may be more useful. All the shear-force data for all cuts and methods available were averaged for all steers in all years (Table 1). Heritability estimates of such average values were 64.48 per cent over-all and 47.84 per cent within breeds and crosses. Part of the difference between sires was accounted for by breed differences, but the major portion was due to differences between sires of the same breed. These values substantiate earlier reports and support the premise that there are differences in tenderness between individuals treated similarly. A significant portion of these differences is due to differences in heredity, and the hereditary portion is subject to simple selection. More research is needed to extend the implications of this work, with confidence, to the general beef-cattle population.

Two major deterrents exist. One is the fact that progeny testing is necessary, since only slaughtered animals can be evaluated. Progeny testing is slow and costly, and simplified techniques are needed. Biopsies may prove useful, but relationships between tenderness and other more easily observable characteristics should be investigated. The chief of the Bureau of Animal Industry (8) reported that superiority observed in the growth of a sire's calves was again evidenced in the form of more tender meat. In the present study the correlation of shear-force value with gain per day of age was -.17 over all breeds;

with gain on test, -.49 over all breeds; and with gain on test within breeds, -.44. The correlation between gain of sires and average shear of progeny was -.43 over-all and -.20 within breeds. At present, data are being gathered at the Texas Station on such characteristics as fineness of hair and temperament as possible leads. Preliminary observations on temperament appear promising.

The second difficulty is the problem of economic recognition for the breeder who may select for tenderness and be successful in attaining his goal. If the trend toward a "meat-type" beef animal develops to the extent that it has in swine, and fat continues to become less popular, the tenderness problem may be accentuated, so that breeding will have to be used as an aid in maintaining or increasing tenderness and offering a more homogeneous product. There is no doubt that the traditional resourcefulness of the beef industry will overcome this last difficulty to the extent justified.

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XIII

PIGMENT-ANTIOXIDANT RELATIONSHIPS TO MEAT-COLOR STABILITY

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The color of meat is highly important to the consumer, and proper color is recognized as a quality attribute of any meat product. The heme pigments responsible for the desired color in various meat products have been the object of considerable research, and many of them have been chemically characterized. Of these, two are of greatest economic importance to the meat packing industry: the pigment of fresh meat, oxymyoglobin, and the cured meat pigment, denatured globin-nitric oxide hemochrome. The discoloration of these and other meat pigments is primarily due to oxidation which, under proper circumstances, is repressed by the naturally occurring antioxidants (tocopherols) and reducing substances (primarily sulfhydryl compounds). In some products the naturally occurring antioxidants and reducing compounds may not have sufficient capacity to prevent the undesirable oxidation. For such cases a number of commercially available materials may be incorporated in, or on, the product in question. Ascorbic acid, including its salt and stereoisomers, is the most important reducing compound in present commercial use.

This report summarizes information relative to the meat pigments and their relationships to antioxidants, particularly as regards color stability. Myoglobin, the muscle pigment, will be used in a general sense in this paper for the heme protein pigment, or derivatives thereof, responsible for meat color. Hemoglobin, the blood pigment, has properties sufficiently similar to make this generalization valid; furthermore, hemoglobin is present in meats in much smaller concentrations than myoglobin. In the figures included herein the basic iron protoporphyrin structure common to all the pigments is shown schematically, with the nitrogen atoms representing the four pyrrole rings.

It should be noted that the basic biochemistry of heme pigments is the object of considerable study aimed at ultimate medical application. The body of basic information available from these researches is of considerable value to the investigator interested in pigment problems in meat processing and preservation (22, 23, 35). The most recent over-all review of the pigments of meats is that of Watts (31).

THE PIGMENTS OF FRESH MEATS

The bright-red pigment of fresh meat is oxymyoglobin, and its stepwise conversion to brown metmyoglobin is the major discoloration problem of fresh meats. The first step in this conversion is the deoxygenation of oxymyoglobin to yield myoglobin. This transformation is readily reversible in an oxygen atmosphere; however, in the interior of meats the respiratory enzymes, particularly succinoxidase, consume oxygen and tend to deoxygenate oxymyoglobin

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to myoglobin, which is purple-red. Myoglobin, with the iron in its heme moiety in the ferrous state, is very labile to oxidation to the brown ferric compound, metmyoglobin. Figure 1 illustrates these reactions.

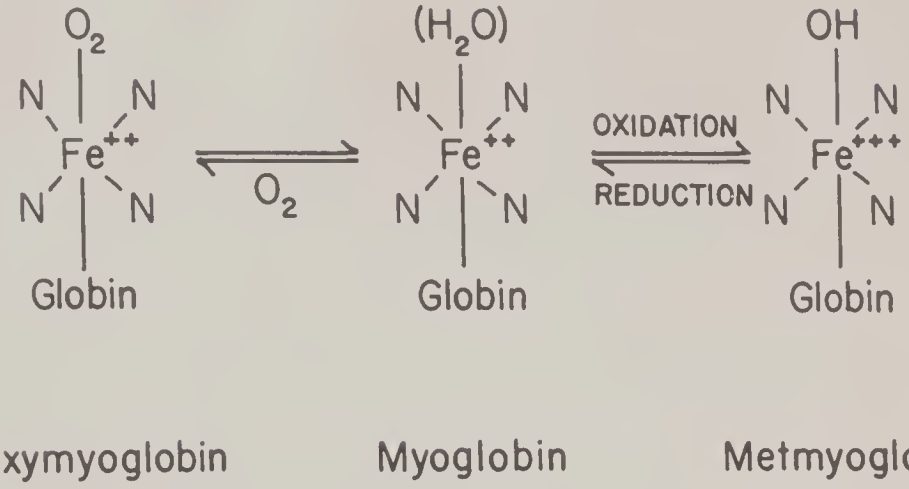

Figure 1. Pigment changes in fresh meats.

The reversible first step, oxymyoglobin-myoglobin transformation, is primarily a function of oxygen availability and is not subject to control by antioxidants; the change is an oxygenation, not an oxidation. Oxymyoglobin is quite stable at high oxygen partial pressures; thus the success of prepackaged meat, insofar as color retention is concerned, is dependent upon use of an oxygen permeable film (21). The oxidation of myoglobin to metmyoglobin, however, is amenable to control; reducing compounds afford protection by continuously regenerating myoglobin from metmyoglobin. In the presence of oxygen the resulting myoglobin is then oxygenated to oxymyoglobin. Ascorbic acid might be used as a reducing compound for the regeneration, although its action in this case is complicated (3, 8, 31).

Considerations of microbiological problems of meat are not in the scope of this paper; it should be mentioned, however, that large bacterial populations may consume oxygen, thus favoring myoglobin formation and subsequent oxidation to metmyoglobin (3, 21). Light does not greatly affect the color of fresh meats; however, exposure of prepackaged meats to ultraviolet light should be avoided, since this favors the myoglobin-metmyoglobin transformation (19). No extensive investigations of the use of reducing compounds incorporated in packaging films have been reported, although the possibility has been mentioned (21). Also of interest are the metmyoglobin reductase enzyme systems of mammalian muscle and blood (23, 35). These enzymes are ill characterized at present, but protection against discoloration of fresh meats might be afforded by providing the environment and reactants necessary for the proper function of these enzymes.

It has been suggested that niacin forms a definite compound with myoglobin and that the addition of niacin to fresh meat may prevent discoloration (31). Our recent studies suggest that, while nicotinamide may protect oxymyoglobin from discoloration, it does not form a compound with oxymyoglobin. It might be expected that nicotinamide would react with myoglobin to form nicotinamide myoglobin, but no evidence for this reaction was found. In pure systems nicotinamide will not reduce methemoglobin; furthermore, the addition of nicotinamide to oxymyoglobin or to the surface of fresh meat produces no change in spectral absorption.

THE PIGMENTS OF COOKED MEAT

When meat is cooked, the globin portion of the myoglobin molecule is denatured, and the iron of the heme is oxidized from the ferrous to the ferric state. The denatured globin co-ordinates with the iron to form denatured globin hemichrome. This substance has been recently identified by its spectral characteristics as the pigment of cooked meats (27). Since the consumer judges the doneness of meat by the presence of this tan pigment, its proper formation is important. It is sometimes noted that a cooked meat turns from tan to pink during storage; this is undoubtedly due to transformation of the tan hemichrome to the pink hemochrome. This change involves the reduction of the ferric iron to the ferrous state and readily occurs under the proper reducing conditions; these conditions apparently occasionally exist in some cooked meats. The reactions involved in the formation and reduction of the cooked-meat pigment are shown in Figure 2. It is of interest that in the case of cooked tuna similar pigments are involved, but the desired compound is the pink hemochrome, while the presence of the tan hemichrome in this product is undesirable (1, 2).

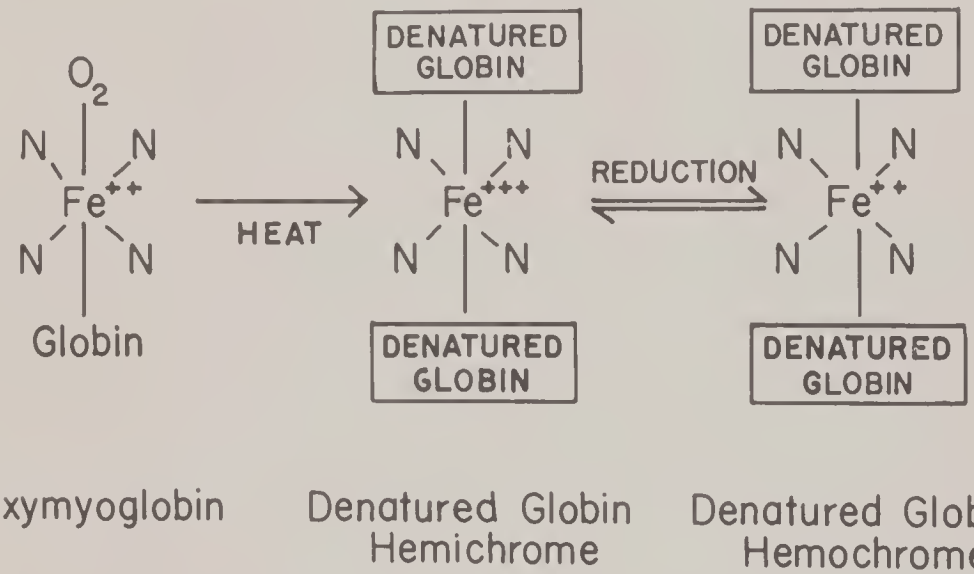

Figure 2. Pigments of cooked meats.

If a meat is exposed to a sufficient concentration of carbon monoxide, as might happen in both gas- and electric-oven cooking, then a carbon monoxide derivative of denatured globin hemochrome can form. This red-colored pigment, denatured globin-carbon monoxide hemochrome, has been noted in poultry and roast meats.

The use of reflectance spectrophotometry is very useful for measurement, identification, and differentiation of the hematin pigments. It is probably the most useful means for studying the non-extractable pigments, such as those of cooked meat, since it can be used for characterization of the pigments without the danger of inducing artifacts through extraction or other chemical manipulation. Figure 3 shows typical reflectance spectra of some meat pigments and illustrates the use of this analytical technique.

THE PIGMENTS OF CURED MEATS

The desired pink color is one of the primary criteria by which cured meats are judged. Antioxidants and reducing agents are required reactants for the proper formation and stabilization of the cured-meat pigment. The meat

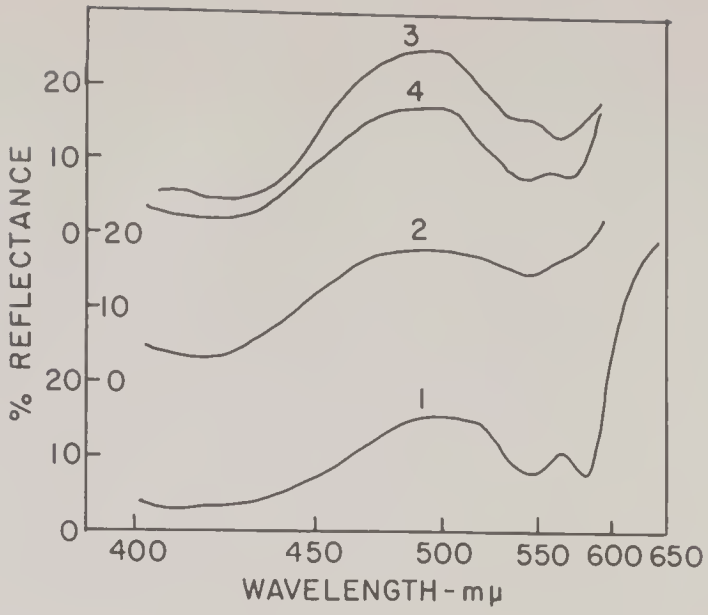

Figure 3. Reflectance spectra of beef. 1, Fresh uncooked beef (oxymyoglobin); 2, Well-done cooked beef (denatured globin hemichrome); 3, Reduced cooked beef (denatured globin hemochrome); 4, Reduced cooked beef treated with carbon monoxide.

pigments must be reacted with nitrite under conditions such that myoglobin is kept reduced and nitric oxide is formed from nitrite. Cellular reducing systems may afford these reducing conditions, or they may be accomplished by the addition of ascorbic acid or other reducing agents. The pigment formed under these circumstances, nitric oxide myoglobin, is relatively unstable and subject to oxidation (13, 24, 30). However, when the product is heated, the more stable cured-meat pigment is formed. This pigment has been identified by spectral characteristics as denatured globin-nitric oxide hemochrome (29). The sulfhydryl groups freed by the denaturation of the protein are of crucial importance to cured-meat color development and stability due to their reducing properties (5, 32). Compounds other than nitrite which are capable of forming hemochromes might someday find application in the meat-processing industry. For example, nicotinamide and histidine might prove to be attractive substitutes for or additions to nitrite, since they are capable of forming similarly colored hemochromes. A major difficulty is that most hemochromes are considerably less stable to oxidation than is denatured globin-nitric oxide hemochrome.

The most important discoloration of cured meats is that resulting from oxidation of the pigment to the brown hemichrome; nitric oxide may be lost in the reaction. This oxidation is favored by the presence of oxygen and light; hence these factors should be controlled as much as possible by proper packaging (4, 14, 15, 16). The reverse reaction leading to the re-formation of the denatured globin-nitric oxide hemochrome may be brought about by the presence of reducing agents and nitrite. Added nitrite is of benefit only if reducing conditions prevail; otherwise it may result in still more rapid fading (32). If no reducing substances are added to the meat, then the desired reducing conditions are dependent upon protein sulfhydryl groups. The loss of such sulfhydryl groups in storage is not greatly affected by conditions of storage other than temperature; higher temperatures cause sulfhydryl loss (5, 34). The most effective reducing agents that can be added to cured meats are ascorbic acid, cysteine, and glutathione. Of these compounds, cysteine and glutathione are not suitable for commercial use due to their high cost; ascorbic acid, however, is widely used in cured meats to help obtain the desired color and increase its stability (12, 17, 33).

In addition to the oxidation of denatured globin-nitric oxide hemochrome to the hemichrome, another type of reaction resulting in discoloration is that leading to oxidation of a methylene bridge in the protoporphyrin structure of the heme. Such oxidations give rise to green verdohemochromes and are brought about by peroxides; the peroxides responsible may be formed as a result of microbial contamination or, less frequently, fat oxidation.

The various transformations discussed are outlined in Figure 4. Reflectance spectra of normal and oxidized cured-meat products are presented in Figure 5. Such spectra are useful for characterization of these pigments; the use of ratios of reflectance or absorption at two specific wave lengths of light has also been proposed (7, 20).

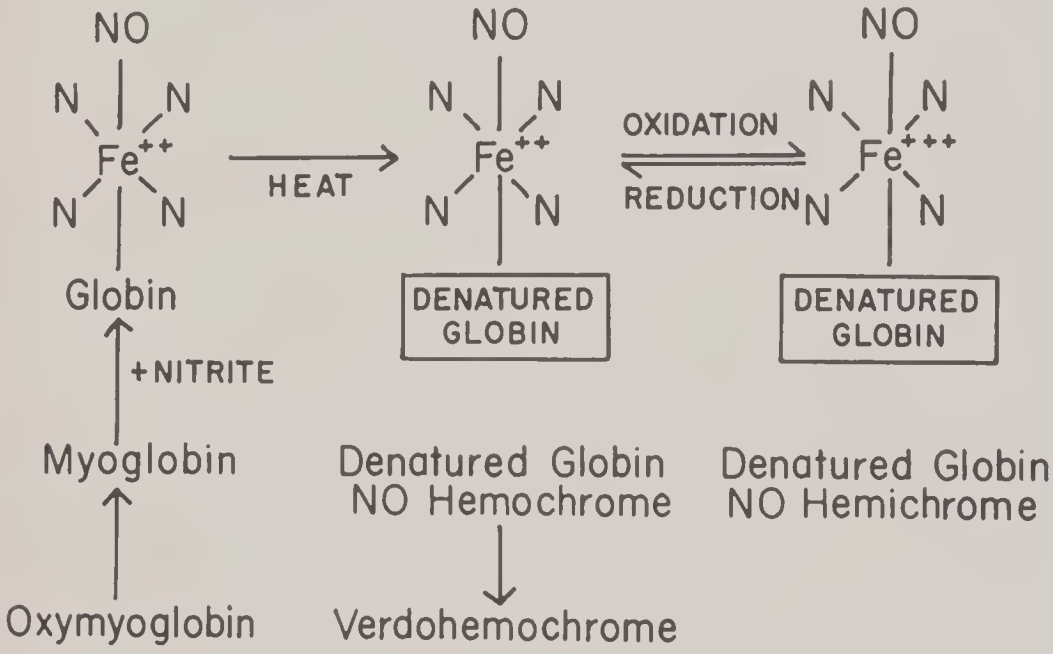

Figure 4. Pigment changes in cured meats.

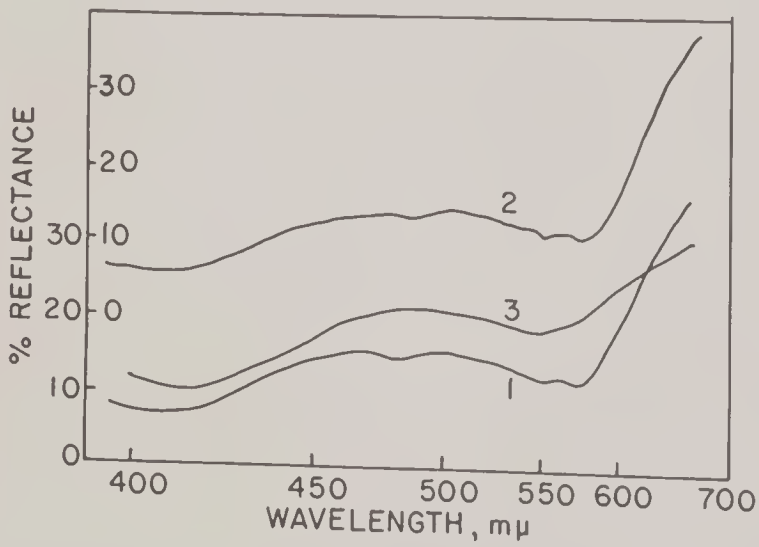

Figure 5. Reflectance spectra of cured meats. 1, Smoked ham; 2, Sandwich beef; 3, Canadian bacon, oxidized.

THE PIGMENTS OF IRRADIATED MEAT

A major question relative to the use of gamma irradiation for sterilization of meat and meat products concerns the effect of such treatment on color. The principal changes in meat pigments due to irradiation are apparently oxidative-reductive in nature. Fresh meats or myoglobin model systems irradiated in an oxygen atmosphere show extensive loss of red color, and in some cases a green pigment is formed. In nitrogen atmospheres, on the other hand, irradiation of fresh meats or myoglobin model systems does not cause pigment destruction but instead regenerates oxymyoglobin. This may be due to interaction of metmyoglobin with free radicals (e.g., hydroxyl radical) produced by the irradiation process (9, 10, 11, 26). The irradiation of cooked meats in nitrogen atmospheres brings about the reduction of denatured globin hemichrome to the pink hemochrome (6, 18, 28). Irradiation of cured meats results in color loss, apparently due to hemichrome formation. Dipping the meats in nitrite plus ascorbate prior to irradiation serves to protect the color; however, the development of rancidity in such cases is pronounced. The irradiation of ground pork treated with a cure containing ascorbate produces a product with a good stable color (6, 11).

THE PIGMENTS OF FREEZE-DRIED MEAT

In meat which is freeze-dehydrated, oxymyoglobin is apparently deoxygenated and remains deoxygenated while the meat is in the dry state. The resulting myoglobin is oxidized to brown metmyoglobin. However, the red color of oxymyoglobin can be regenerated by reducing agents in the presence of oxygen during or after rehydration. Either carbon monoxide or nitric oxide could be used to form the pink hemochrome which stores well in this product (25).

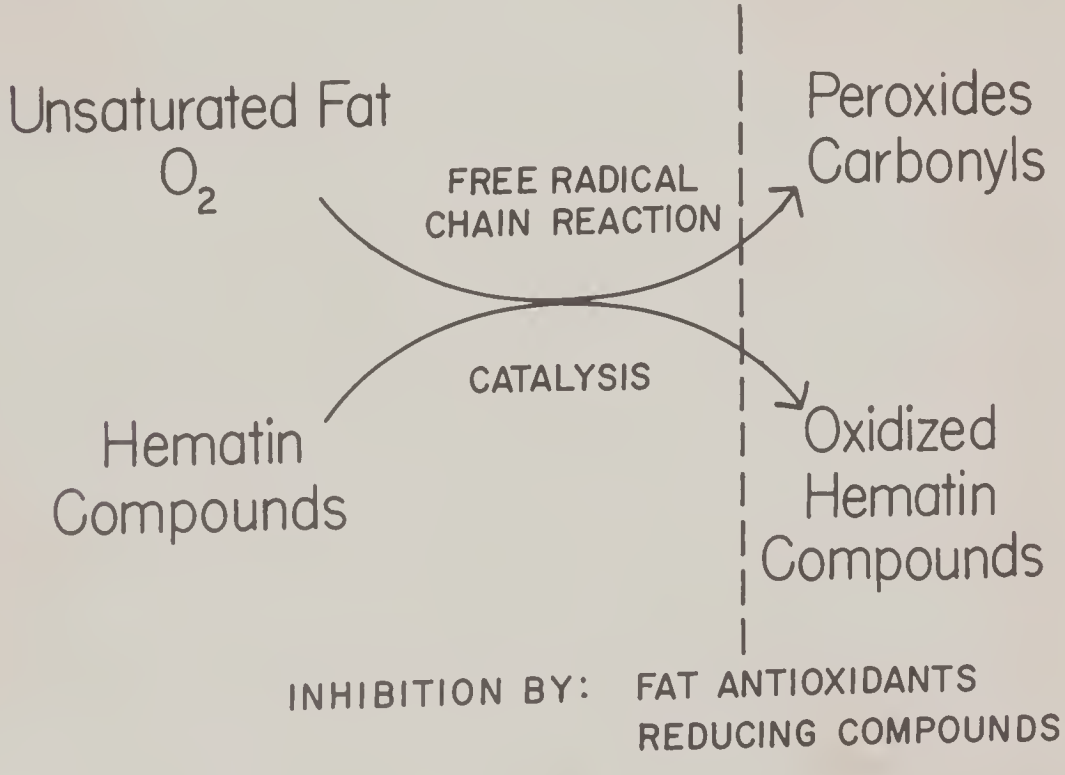

Figure 6. Oxidative fat rancidity and pigment changes.

OXIDATIVE FAT RANCIDITY AND PIGMENT LOSS IN MEAT

The development of oxidative fat rancidity has been shown to limit the storage life of frozen poultry and fish and some meat products such as frozen pork. In meats the hematin compounds are the dominant catalysts of the free-radical chain reaction oxidation of unsaturated fats to form peroxides and subsequently carbonyls which are responsible for the rancidity. Concurrently, the hematin compounds may react with the free radicals produced, resulting in degradation of the iron protoporphyrin and loss of hematin compound color. Both of these undesirable reactions, oxidative fat rancidity and destruction of the hematin pigments, can be inhibited by fat antioxidants and reducing compounds. In the presence of fat antioxidants, reducing compounds act synergistically to enhance their effectiveness. These reactions are schematically represented in Figure 6.

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XIV

SOME FACTORS INFLUENCING THERMAL RESISTANCE OF BACTERIAL SPORES

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The resistance of bacterial spores to destruction by heat is of concern to all who process foods by thermal procedures. The thermal-resistance characteristics of the spores which are to be destroyed dictate the process conditions for the food being prepared. In some foods the thermal-process conditions required are so severe that they contraindicate the canning of a particular food product. When a thermal process is employed, the process frequently causes a greater destruction of the organoleptic and nutritive properties of the food than what is desired for the greatest acceptance of the product. The process conditions used are such that they assure the destruction of food-poisoning organisms, such as the spores of Clostridium botulinum, and seek to destroy the spores of all the food-spoilage types which might be present in the product. In most cases the process conditions employed are based on scientific and technical information on the thermal-resistance characteristics of the spores that are to be destroyed.

Our scientific literature contains numerous reports which describe the thermal-resistance properties of the spores of a given organism under particular conditions or in a given food product. This information has been very useful because it has assured the production of safe canned foods that have developed a minimum amount of bacterial spoilage. In contrast to the more practical data just mentioned, our scientific literature contains relatively little information which explains why spores are so resistant to heat or which describes the changes which occur in the spores during destruction by heat. Information on both these aspects would be helpful to us in understanding the death reaction and would ultimately be of practical value to the food processor.

FACTORS WHICH AFFECT THE HEAT RESISTANCE OF SPORES

The spores of various species of bacteria vary greatly in their resistance to destruction by heat. We may consider these differences to be inherent properties of the spore, and they are reflected in strain differences as well as in species differences. The data of Murrell (1) demonstrate the wide difference in thermal resistance exhibited by the spores of a variety of bacteria (Table 1). Inspection of these data indicates that the time required for the destruction of 99 per cent of the spores, at 250°F., ranges from 0.0003 minute for Clostridium botulinum Type E to 28 minutes for Bacillus stearothermophilus. This is almost a 100,000-fold difference in thermal destruction time.

The resistance of spores of a particular strain of an organism may also be varied. These differences are related to the nutritional and cultural conditions of the cell during sporogenesis. We will comment on some of these effects, as it is quite probable that a further study of this aspect of spore thermal

TABLE 1

The Approximate Heat Resistance of the Spores of Some Organisms in Phosphate Buffer (pH 7)*

Organism	99 Per Cent Destruction Time† (Min., 250° F.)
Bacillus cereus	0.013
B. megatherium	0.08
B. subtilis	0.16
Bacillus species (Strain 320)	6
B. stearothermophilus	28
Clostridium botulinum, Type E	0.0003
Cl. bifermentans	0.12
Cl. botulinum, Type A (Strain 62)	0.24
Cl. botulinum, Type B (Strain 213)	0.36
Cl. sporogenes (P.A. 3679)	3
Cl. thermosaccharolyticum (Strain 3814)	8

*From Murrell (1).

† Destruction times at experimental temperature converted to the equivalent at 250° F. (121.1° C.) assuming a temperature coefficient of 10 throughout.

resistance will give us information which will be helpful in understanding the mechanism of heat resistance. Various investigators (2, 3) have demonstrated that the thermostability of spores of a particular organism increased as the incubation temperature of sporulation was increased. Some of our own data which substantiate this observation are presented in Table 2. If we consider

TABLE 2

Effect of Sporulation Temperature on Thermal Death Rate of Spores of Bacillus coagulans*

Sporulation Temperature (°C.)	$\underline{D}$†	12 $\underline{D}$ (Min.)
30	4.4	52.8
37	11.0	132.0
45	20.0	240.0

*Spores were heated in M/40 phosphate buffer, pH 7 at 93°C.

† $\underline{D}$ = time in minutes for a 90 per cent destruction of the heated spores; average of four determinations.

that 12 D values are required to sterilize the product at our test temperature, you will note that the sterilization time increases from about 53 minutes for spores produced at 30°C. to 240 minutes for those that were produced at 45°C. Unfortunately, no one has examined spores produced at such different temperatures for specific differences in spore composition. This wide difference in thermal resistance must be somehow related to the biochemical structure or composition of the spores. Even an incomplete characterization should be helpful in explaining the thermal-resistance properties of these bacterial spores and should give us an insight as to why spores are so resistant to heat.

An important difference in composition between vegetative cells and spores was found by Curran et al. (5), who demonstrated that spores contained more calcium than did the corresponding vegetative cells. Sugiyama (3) presented evidence that the thermostability of Clostridium botulinum spores was dependent on the calcium and iron content of the sporulation medium. Media which were deficient in these cations produced spores of reduced thermostability.

It was later found that, when the incubation temperature of sporulation was kept constant, the thermal resistance of the spores could be altered by changing the composition of the sporulation medium. El-Bisi and Ordal (4) reported that the phosphate concentration of the sporulation medium markedly affected the thermostability of spores of Bacillus coagulans. They presumed that spores produced on media containing high concentrations of phosphate were of reduced thermostability because the excess phosphate in the media rendered necessary cations, such as calcium, unavailable to the sporulating cells. Amaha and Ordal (6) substantiated these observations and demonstrated that sporulation media fortified with calcium and manganese yielded spore harvests possessing an increased thermostability.

In this connection it should be emphasized that certain minerals are required for the production of normal spores. With the aerobic spore-formers, the addition of small quantities of manganese markedly stimulates sporulation (7, 8).

These few remarks strongly imply that the thermostability of bacterial spores is somehow related to the cation content of the spores and possibly in the way that these cations are bound to the other spore material. That this is reasonable is supported by the data the biochemists have provided us on the effect of various cations, such as calcium, on the thermostability of proteins (9, 10).

Bacterial spores are rich in a unique compound which so far has not been detected in vegetative cells. This compound is 2,6-dicarboxypyridine, or dipicolinic acid (DPA) (11) (Fig. 1). In the spores so far examined it has been found in amounts ranging from 5 to 15 per cent of the dry weight of the spores. The presence of such large amounts of DPA in the spore coupled with the fact that it is absent in vegetative cells strongly suggests that it is also somehow

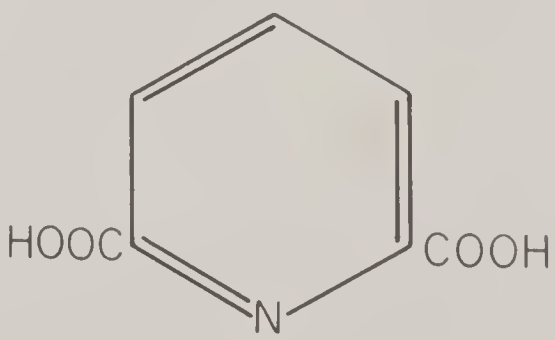

Figure 1. Dipicolinic acid (DPA).

related to their thermostability. While current available data are fragmentary, it does not appear that there is a direct correlation between the amount of DPA in the spores and their degree of thermostability. In fact, spores of different species of bacteria may contain similar amounts of DPA but vary widely in their resistance to thermal destruction. However, it is conceivable that the way the DPA is incorporated into the spore material may also affect its thermostability. This may then explain why spores of similar DPA content have different degrees of resistance to thermal destruction or why spores with a lower DPA content are more resistant to thermal destruction than spores of other species which normally contain a greater amount of DPA.

A more detailed study of the death reaction per se and of the changes which occur in the spore during destruction by heat should yield information which would be helpful in analyzing the over-all problem. We have been carrying on some investigations in this area, and I would like to present some of our results. The work is as yet incomplete, so what I will tell you is more or less a progress report, with the interpretations of the results tentative.

CHANGES IN THE BACTERIAL SPORE DURING DESTRUCTION BY HEAT

It has been a common hypothesis that death, produced by moist heat, proceeds at an exponential rate and, therefore, that the reaction itself is similar to that of a first-order reaction (12). This has been one of the basic assumptions in our thermal-process calculations and as such has served us well. However, despite the extensive amount of work that has been done and is being done on the thermal destruction of bacterial spores, there is relatively little published material on the death reaction itself or on the changes which occur in the spore during thermal death.

Our studies on the death of the spore have utilized, as a test organism, Bacillus coagulans var. thermoacidurans. The spores were prepared by producing them on a modified thermoacidurans agar which contained 0.5 per cent proteose peptone, 0.5 per cent yeast extract, 0.5 per cent glucose, 0.05 per cent monobasic potassium phosphate, 1 p.p.m. manganese sulfate, and 2 per cent agar. The pH was adjusted to 6.8. After harvest, the spore crops were washed once, resuspended in 0.1 per cent KCl, and then subjected to a lysozyme treatment. We have found that the residual sporangia and vegetative debris in spore harvests were readily solubilized by the action of this enzyme. Following the enzyme treatment, these spores are washed from eight to ten times with cold distilled water, using centrifugal procedures. The resulting spore preparations appeared to be free of all extraneous material, and the spores readily resuspended when they were shaken in distilled water. When an aliquot of such a spore preparation was heated at 98.5°C. in M/40 phosphate buffer at pH 7.0, the survivor curve presented in Figure 2 was obtained. It is apparent that these data do not reflect conditions for a typical first-order reaction but rather that during the first 40-45 minutes the rate of death is accelerating and then establishes itself at a constant rate. We might say that more than one event must occur before the spore is killed or that a series of reactions must take place before the more marked effect of this temperature can manifest itself. One might also argue that this curve reflects the degree of spore-clumping in our reaction mixture. However, we have been unable to detect evidence of clumping, and the data are quite reproducible not only for duplicate determinations on the same spore harvest but also for determinations on replica spore harvests. For comparative purposes we have included a hypothetical curve in which we assumed that the death rate was exponential and that it proceeded at a rate equal to that of the second part of the actual survivor

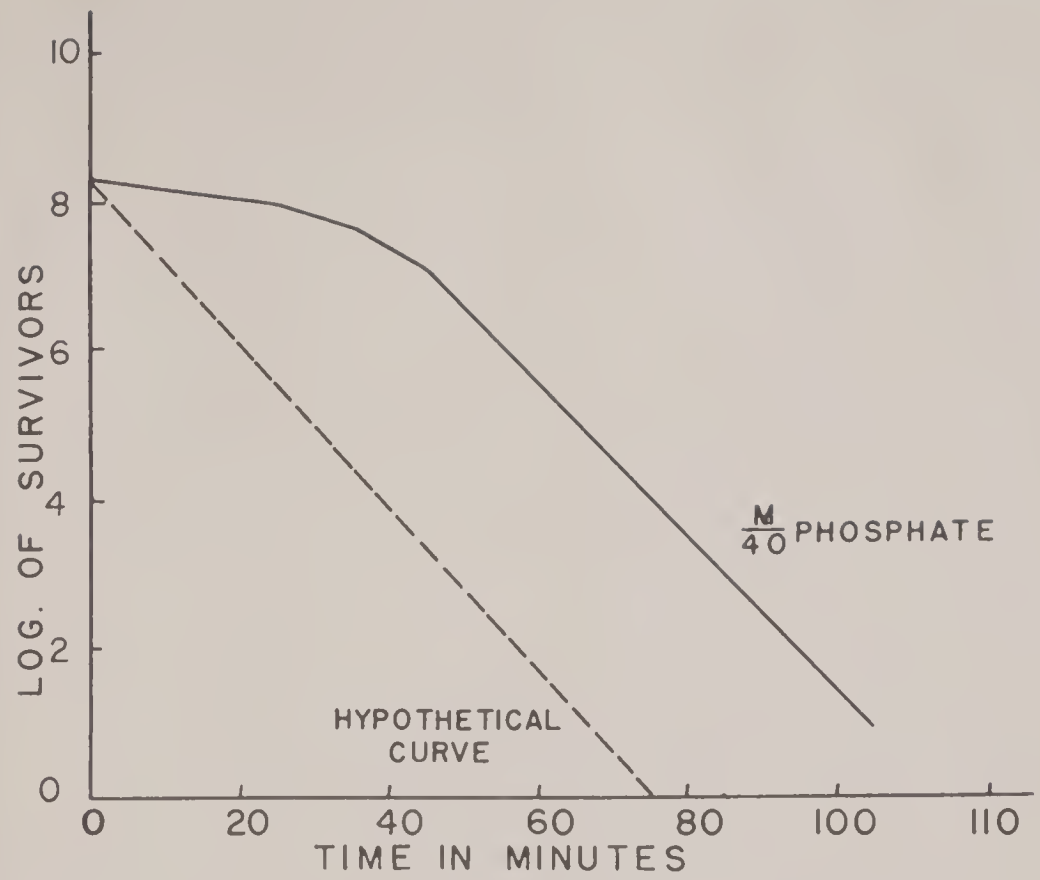

Figure 2. Survivor curve for spores heated in M/40 phosphate.

curve. If we assume that the thermal death time at this temperature is equal to the intercepts of the curves with the abscissa, the differences in thermal death time would be pronounced.

The shape and the position of the survivor curve can also be changed even though the temperature and pH are kept constant by changing the composition of the heating menstruum. It is necessary to present these data graphically, as survivor curves, since it is impossible to choose a death-rate constant, such as the D value, which truly represents the rate or changes in rate of the death reaction.

We have previously discussed the apparent relationship between the mineral content, especially calcium and manganese, of spores and their thermostability. We will now proceed to a further examination of the effect of the phosphate concentration in the heating menstruum on the death rate of Bacillus coagulans var. thermoacidurans spores. Representative data are presented in Figure 3. An inspection of these data demonstrates that maximum resistance to thermal destruction occurred when the spores were suspended in M/40 phosphate buffer. When the phosphate concentration was either increased or decreased, the death reaction was accelerated. Our tentative hypothesis for this twofold effect was that the higher concentrations of phosphate increased the death rate because of the affinity of the phosphate anion for various cations and thus accelerated their release from the spore. In the case of the lower phosphate concentrations, we presumed that the low ionic strength of the heating menstruum accelerated the death reaction because of osmotic and ion-concentration differences between the spore and the heating menstruum.

Results in support of the latter part of this hypothesis are furnished by the data obtained in another series of experiments. In these experiments we used M/200 phosphate to furnish enough buffer capacity to maintain the pH constant during heating. We then raised the ionic strength of this solution equal to that of M/40 phosphate by the addition of suitable amounts of various salts, such

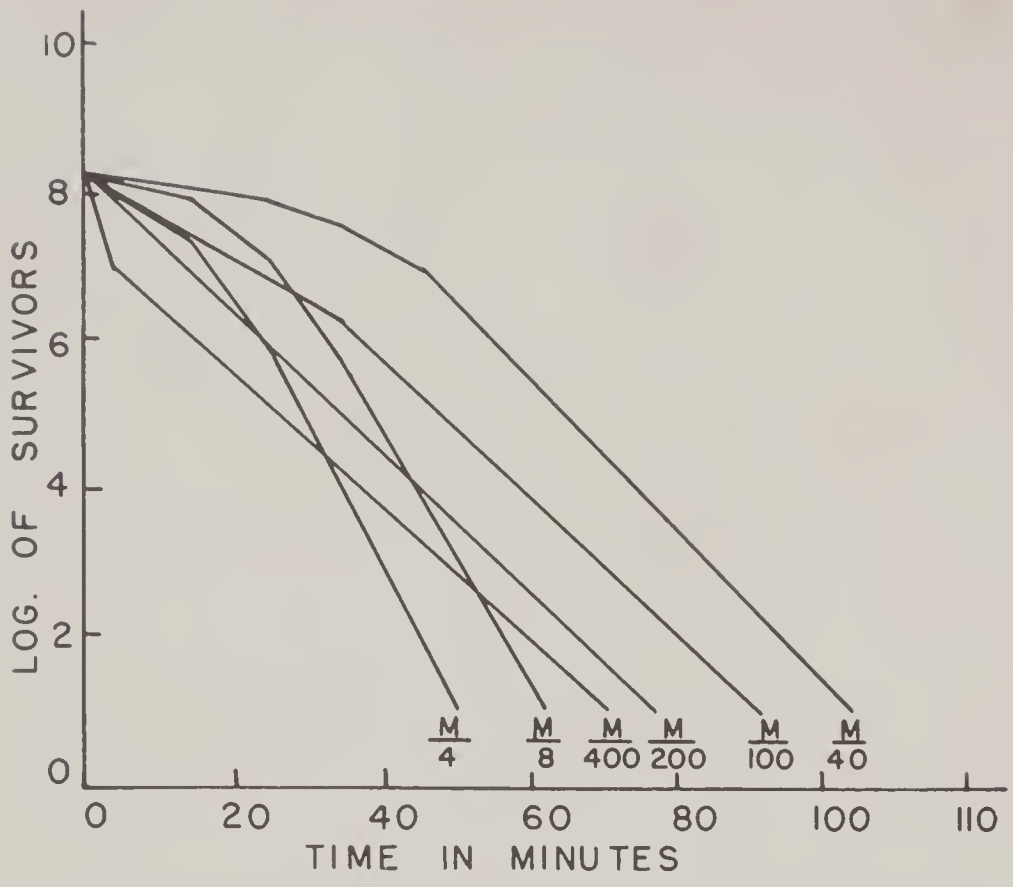

Figure 3. Effect of phosphate concentration on thermal resistance.

as NaCl, KCl, $NaSO_4$, and Na_2NO_3. The thermal death rate was followed in each of these mixed solutions. The survivor curves so obtained were superimposable on the curve for our reference condition, namely, M/40 phosphate buffer. These results, therefore, imply that the ionic strength of the heating menstruum may affect the death rate of these heated spores and that the rate is accelerated in environments of low ionic strength.

Support for the first part of our hypothesis is more indirect and comes from the observations that the death-rate reaction is accelerated in the presence of various chelating agents. The results of some typical experiments using phosphate buffer, tris buffer (trihydroxymethylaminomethane), EDTA (ethylenediaminetetraacitic acid), and glycylglycine (GG) are presented in Figure 4. In this experiment we compared the death of our spores in M/100 solutions of the four different substances. The pH in each case was adjusted to 7.0, and the reaction followed at 98.5°C. The survivor curve for the same spores in M/40 phosphate buffer is included for comparison. Each substance produces its own characteristic effect, and, while these results do not tell us which ion or ions are the most important with respect to the thermostability of the spore, they do suggest that conditions which favor the removal of cations also accelerate the death reaction.

The discussion so far has indicated that the death reaction is a complex one and that it is markedly affected by the heating environment. It would be desirable to correlate some of the above conditions with some of the changes which occur in the spore during thermal destruction.

When heated spores were examined under the phase microscope, there seemed to be no dramatic differences in their appearance in comparison to unheated spores. There was a trend toward a loss of refractility, but the change was by no means so pronounced as the differences between germinated and ungerminated spores. Likewise, the increase in the stainability of air-dried smears was quite minor. However, excessive heating such as autoclaving

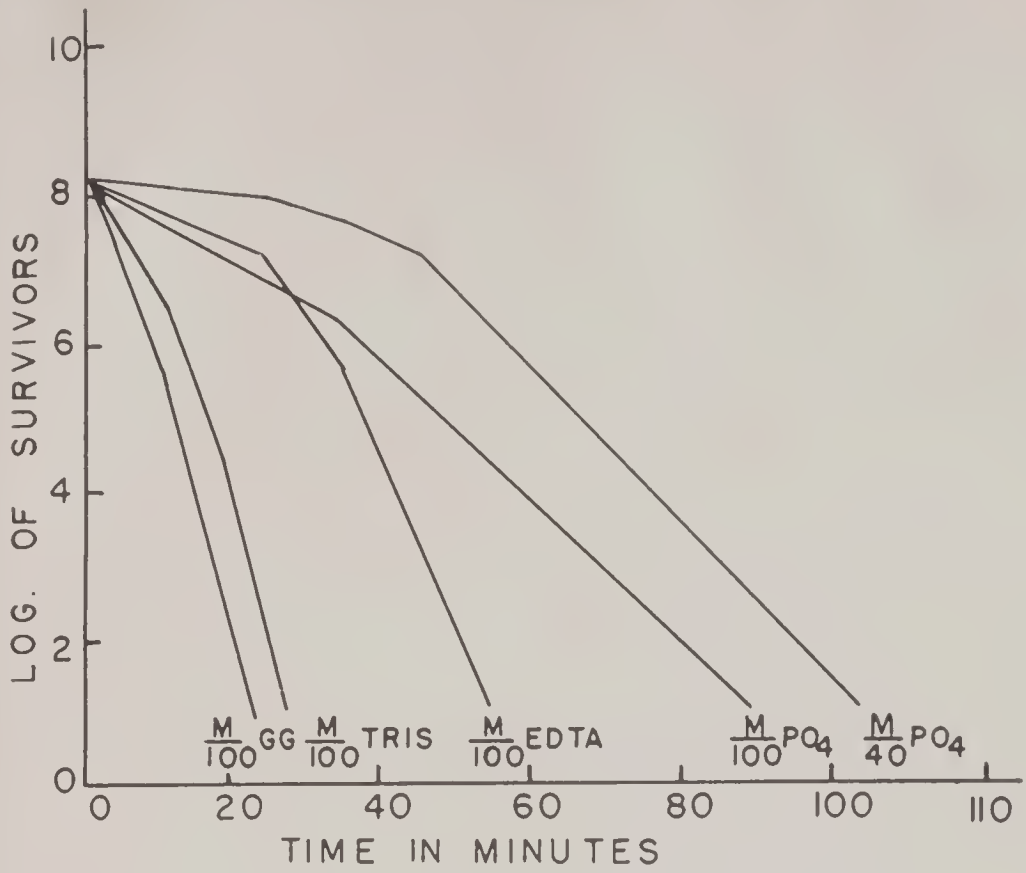

Figure 4. Effect of composition of heating menstruum on thermal resistance.

did cause a loss of refractility and an increase in stainability. Excessive autoclaving caused a disruption of the spore.

In contrast to these apparently minor morphological changes, rather striking chemical changes take place during thermal destruction. Various investigators have demonstrated that, during spore germination, the DPA was rapidly released from the spore and appeared in the supernatant liquid (11). Similarly, during thermal death, the DPA of the spore was released into the heating menstruum (13). An investigation of the rate of release of DPA from spores showed that, when spores of different thermostabilities were heated under comparable conditions, the DPA of the more heat-sensitive spores was released more rapidly than that of the more heat-resistant spores (Amaha and Ordal, unpublished results). In another experiment in which spores of a given heat resistance were heated in a menstruum which accelerates the death reaction, the rate of the DPA release was also accelerated (Lechowich and Ordal, unpublished results). Some of the changes which accompanied the release of DPA during thermal death are presented in Table 3. In this experiment concentrated spore suspensions were heated in water and in M/100 glycylglycine. The death reaction was followed by conventional plating procedures; the DPA release, by the procedure of Janssen et al. (13); and the release of the cations, calcium, manganese, and magnesium, was determined with the aid of an emission spectrograph. In the chemical determinations the amounts found in the supernatant liquid were compared with what remained in the sedimented spores.

These data indicate that the thermal destruction of bacterial spores is accompanied by definite changes in the composition of the spore. While they were not complete enough to establish suitable rate curves for each of the constituents which were analyzed, the data do support the hypothesis that some of them are related to the thermal-resistance characteristics of the spore. Only a small proportion of the total magnesium content was found in the supernatant even

though nearly all the spores were destroyed. One assumption would be that magnesium is not directly related to thermostability. The rather large proportion of DPA, calcium, and manganese released implies that they are more intimately related to the thermoresistance properties of the spore. The reason for the difference in the amount of DPA released between those heated in water and those heated in glycylglycine is not apparent at this time. In other experiments we have demonstrated that, by the time the spores were destroyed, essentially all the DPA of the spore could be found in the supernatant liquid. The amount of calcium released is quite comparable in the two heating menstrua. It is tempting to speculate that the greater release of manganese from the spores heated in the glycylglycine solution is the reason for the more rapid death rate in this menstruum. However, this must be substantiated by further experimentation.

TABLE 3

Changes in Bacterial Spores Following Thermal Destruction

Property Analyzed for	Per Cent of Total Destroyed or Released	
	H_2O*	GG†
Spores destroyed	99.7	99.9
Dipicolinic acid (DPA)	93	77
Calcium	45	50
Manganese	66	97
Magnesium	12	15

*After 65 minutes heating in water at about 100°C.

†After 25 minutes heating in M/100 glycylglycine at about 100°C.

Further studies should also be made on other conditions which alter the thermal-resistance properties of a given species of spores and the consequent changes which occur in such spores during thermal death. A correlation of the data so obtained would provide us with a better understanding of why bacterial spores are so resistant to heat and would present us with a more adequate background for evaluating a given thermal process for a particular food product.

ACKNOWLEDGMENT

We are indebted to Dr. H. V. Malmstadt and Mr. A. V. Schalge, of the Department of Chemistry, for the emission spectrograph determinations of calcium, magnesium, and manganese reported in Table 3.

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